

10/04, 326

? ds

Set	Items	Description
S1	77197	T(W) CELL(W) RECEPTOR??
S2	19632	ZIPPER
S3	185	S1 AND S2
S4	158	S3 AND PY<=1998
S5	418046	SOLUBLE
S6	26	S4 AND S5
S7	19	RD (unique items)
S8	245	SOLUBLE(5N) (T(W) CELL(W) RECEPTOR)
S9	356655	LIGAND
S10	47	S8 AND S9
S11	29	RD (unique items)
S12	19	S11 AND PY<=1998

? s mhc

S13 101598 MHC

? s s8 and s13

245 S8

101598 S13

S14 83 S8 AND S13

? s s14 and py<=1998

Processing

83 S14

33643774 PY<=1998

S15 62 S14 AND PY<=1998

? rd

>>>Duplicate detection is not supported for File 340.

>>>Records from unsupported files will be retained in the RD set.

...examined 50 records (50)

...completed examining records

S16 42 RD (unique items)

? s bind? or interact?

Processing

1934387 BIND?

1843653 INTERACT?

S17 3365401 BIND? OR INTERACT?

? s s16 and s17

42 S16

3365401 S17

S18 29 S16 AND S17

? t s18/3,k,ab/1-29

18/3,K,AB/1 (Item 1 from file: 155)

DIALOG(R) File 155:MEDLINE(R)

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11435532 98318213 PMID: 9655481

Detection of antigen-specific T cells with multivalent soluble class II

MHC covalent peptide complexes.

Crawford F; Kozono H; White J



? ds

Set	Items	Description
S1	72576	T(W) CELL(W) RECEPTOR
S2	15773	HETERODIMERI?
S3	340	S1 AND S2
S4	903386	REVIEW
S5	11	S3 AND S4
S6	8	RD (unique items)

? s soluble

S7 418894 SOLUBLE

? s s3 and s7

340 S3

418894 S7

S8 57 S3 AND S7

? rd

>>>Duplicate detection is not supported for File 340.

>>>Records from unsupported files will be retained in the RD set.

...examined 50 records (50)

...completed examining records

S9 40 RD (unique items)

? t s9/3,k,ab/1-40

9/3,K,AB/1 (Item 1 from file: 155)

DIALOG(R) File 155:MEDLINE(R)

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14976739 22340338 PMID: 12454477

The production, purification and crystallization of a soluble heterodimeric form of a highly selected T-cell receptor in its unliganded and liganded state.

Clements Craig S; Kjer-Nielsen Lars; MacDonald Whitney A; Brooks Andrew G; Purcell Anthony W; McCluskey James; Rossjohn Jamie

The Protein Crystallography Unit, Department of Biochemistry and Molecular Biology, School of Biomedical Sciences, Monash University, Clayton, Victoria 3168, Australia.

Acta crystallographica. Section D, Biological crystallography (Denmark)

Dec 2002, 58 (Pt 12) p2131-4, ISSN 0907-4449 Journal Code: 9305878

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

T-cell antigen receptors (TcRs) are heterodimeric cell-surface receptors that play a pivotal role in the cellular immune response. The TcR interacts specifically with a peptide-laden major histocompatibility complex (pMHC). A human TcR has been characterized that interacts with an immunodominant epitope, FLRGRAYGL, from the Epstein-Barr virus, a ubiquitous human pathogen, in complex with HLA-B8. Despite the vast TcR repertoire, this TcR is found in up to 10% of the total T-cell population in seropositive HLA-B8+ individuals. In this report, this highly selected TcR is characterized by expressing in Escherichia coli, refolding, purifying and crystallizing the receptor. In addition, the HLA-B8-FLRGRAYGL complex has been expressed in E. coli, refolded and shown to be functionally active. Using native gel electrophoresis, the refolded TcR is shown to be capable of binding sp



Dialog Acc No: 10175454 IFI Acc No: 2002-0119149 IFI Acc No: 2002-0030365  
Document Type: C

MULTIVALENT **T CELL RECEPTOR** COMPLEXES; CAN BE USED FOR  
DELIVERING THERAPEUTIC AGENTS OR FOR DETECTING MAJOR HISTOCOMPATIBILITY  
COMPLEX (MHC)-PEPTIDE COMPLEXES

Inventors: Boulter Jonathan Michael (GB); Jakobsen Bent Karsten (GB)

Assignee: Unassigned Or Assigned To Individual

Assignee Code: 68000

Publication (No,Date), Applic (No,Date):

US 20020119149 20020829 US 2001912787 20010725

Publication Kind: A1

Division Pub(No),Applic(No,Date): PENDING

US 99334969

19990617

Priority Applic(No,Date): GB 98107592 19980519; GB 98211295 19980929;

WO 99GB1583 19990519

Abstract: The present invention relates to a synthetic multivalent **T cell receptor** complex for binding to a MHC-peptide complex, which multivalent **T cell receptor** complex comprises a plurality of T cell receptors specific for the MHC-peptide complex. It is preferred that the T cell receptors are refolded recombinant **soluble** T cell receptors. The synthetic multivalent **T cell receptor** complex can be used for delivering therapeutic agents or for detecting MHC-peptide complexes, and methods for such uses are also provided.

MULTIVALENT **T CELL RECEPTOR** COMPLEXES...



Dialog Acc No: 10198684 IFI Acc No: 2002-0142389 IFI Acc No: 2002-0036664  
Document Type: C

**SOLUBLE T CELL RECEPTOR; RECEPTOR PROTEIN FOR USE**

AS TOOL IN THE DIAGNOSIS AND TREATMENT OF INFECTION AND AUTOIMMUNE DISEASE

Inventors: Bell John Irving (GB); Boulter Jonathan Michael (GB); Gao George  
Fu (US); Jakobsen Bent Karsten (GB); Willcox Benjamin Ernest (GB)

Assignee: Unassigned Or Assigned To Individual

Assignee Code: 68000

Publication (No,Date), Applic (No,Date):

US 20020142389 20021003 US 200114326 20011113

Publication Kind: A1

Continuation Pub(No),Applic(No,Date): ABANDONED

US 99335087

19990617

Section 371 Pub(No,Date),Applic(No,Date):US 99335087 19990617;WO

99GB1588 19990519

Priority Applic(No,Date): GB 98107592 19980519; GB 98211295 19980929

Abstract: The present invention relates to a recombinant **soluble**

**T cell receptor**. The **T cell receptor**

(TCR) is refolded and comprises a recombinant TCR alpha or gamma chain extracellular domain having a first heterologous C-terminal dimerisation peptide; and a recombinant TCR beta or delta chain extracellular domain having a second C-terminal dimerisation peptide which is specifically **heterodimerised** with the first dimerisation peptide to form a **heterodimerisation** domain, which may be a coiled coil domain. The invention also provides nucleic acid sequences encoding the recombinant TCR and a method for producing the recombinant TCR. The TCR may be labelled with a detectable label so as to enable the detection of specific MHC-peptide complexes. Alternatively, it can be linked to a therapeutic agent such as a cytotoxic agent or an immunostimulating agent so as to deliver such an agent to the site of a specific MHC-peptide complex.



9/3,K,AB/28 (Item 10 from file: 34)  
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci  
(c) 2004 Inst for Sci Info. All rts. reserv.

03626496 Genuine Article#: PU285 Number of References: 25

Title: A GENERAL-METHOD FOR FACILITATING **HETERODIMERIC** PAIRING  
BETWEEN 2 PROTEINS - APPLICATION TO EXPRESSION OF ALPHA-T-CELL AND  
BETA-T-CELL RECEPTOR EXTRACELLULAR SEGMENTS (Abstract Available)

Author(s): CHANG HC; BAO ZZ; YAO Y; TSE AGD; GOYARTS EC; MADSEN M; KAWASAKI E; BRAUER PP; SACCHETTINI JC; NATHENSON SG; REINHERZ EL

Corporate Source: HARVARD UNIV,SCH MED,DANA FARBER CANC INST,IMMUNOBIOLOG LAB,44 BINNEY ST/BOSTON//MA/02115; HARVARD UNIV,SCH MED,DEPT MED/BOSTON//MA/02115; HARVARD UNIV,SCH MED,DEPT PATHOL/BOSTON//MA/02115; YESHIVA UNIV ALBERT EINSTEIN COLL MED,DEPT MICROBIOL & IMMUNOL/BRONX//NY/10461; YESHIVA UNIV ALBERT EINSTEIN COLL MED,DEPT BIOCHEM/BRONX//NY/10461; YESHIVA UNIV ALBERT EINSTEIN COLL MED,DEPT CELLBIOL/BRONX//NY/10461; PROCEPT INC/CAMBRIDGE//MA/02139

Journal: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, 1994, V91, N24 (NOV 22), P11408-11412

ISSN: 0027-8424

Language: ENGLISH Document Type: ARTICLE

Abstract: Generation of **soluble T-cell receptor**

(TCR) molecules by a variety of genetic engineering methods has been hampered by inefficient pairing of alpha and beta subunits in the absence of their respective transmembrane regions and associated CD3 components. To overcome this obstacle, we have added 30-amino acid-long segments to the carboxyl termini of alpha and beta extracellular domains via a cleavable flexible linker. These peptide segments (BASE-p1 for alpha and ACID-p1 for beta) have been previously shown to selectively associate to form a stable **heterodimeric** coiled coil termed a leucine zipper. Homodimeric structures are not permitted due to electrostatic repulsion among amino acid side chains. Expression of a representative TCR-leucine zipper fusion protein in a baculovirus expression system results in production of alpha beta TCR heterodimer at 0.6-1.4 mg/liter. This yield is 5- to 10-fold greater than that of the TCR expressed in the absence of the synthetic leucine zipper sequence. The structure of the TCR component of the fusion heterodimer was judged to be native when probed with a panel of 17 mAbs specific for alpha and beta constant and variable domains. A mAb specific for the isolated BASE-p1/ACID-p1 coiled coil was also generated and shown to react with the TCR fusion protein. The above technology should be broadly useful in the efficient production and purification of TCRs as well as other **heterodimeric** proteins.

Title: A GENERAL-METHOD FOR FACILITATING **HETERODIMERIC** PAIRING  
BETWEEN 2 PROTEINS - APPLICATION TO EXPRESSION OF ALPHA-T-CELL AND  
BETA-T-CELL RECEPTOR EXTRACELLULAR SEGMENTS

Abstract: Generation of **soluble T-cell receptor**

(TCR) molecules by a variety of genetic engineering methods has been hampered by inefficient pairing...

...ACID-p1 for beta) have been previously shown to selectively associate to form a stable **heterodimeric** coiled coil termed a leucine zipper. Homodimeric structures are not permitted due to electrostatic repulsion ...

...be broadly useful in the efficient production and purification of TCRs as well as other **heterodimeric** proteins.

...Research Fronts: THE AUTOGRAPH-A-CALIFORNICA NUCLEAR POLYHEDROSIS-VIRUS P10 GENE; RECOMBINANT JUVENILE-HORMONE ESTERASE)

92-3772 001 (T-CELL RECEPTOR; LPR MICE; EARLY FETAL

THYMOCYTES EXPRESSING FC-GAMMA-RII/III CONTAINS PRECURSORS)

92-4628 001 (T-CELL RECEPTOR V-BETA GENE USAGE;



SIMILAR MYELIN BASIC-PROTEIN PEPTIDE MAJOR HISTOCOMPATIBILITY  
COMPLEXES; CHEMICAL DETAILS OF...

9/3,K,AB/29 (Item 11 from file: 34)  
DIALOG(R) File 34:SciSearch(R) Cited Ref Sci  
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03275916 Genuine Article#: NR989 Number of References: 66  
Title: B7 AND INTERLEUKIN-12 COOPERATE FOR PROLIFERATION AND  
INTERFERON-GAMMA PRODUCTION BY MOUSE T-HELPER CLONES THAT ARE  
UNRESPONSIVE TO B7 COSTIMULATION (Abstract Available)  
Author(s): MURPHY EE; TERRES G; MACATONIA SE; HSIEH CS; MATTSON J; LANIER L  
; WYSOCKA M; TRINCHIERI G; MURPHY K; OGARRA A  
Corporate Source: DNAX RES INST MOLEC & CELLULAR BIOL INC,901 CALIF  
AVE/PALO ALTO//CA/94304; DNAX RES INST MOLEC & CELLULAR BIOL INC/PALO  
ALTO//CA/94304; WASHINGTON UNIV,SCH MED,DEPT PATHOL/ST LOUIS//MO/63110;  
WISTAR INST ANAT & BIOL/PHILADELPHIA//PA/19104  
Journal: JOURNAL OF EXPERIMENTAL MEDICINE, 1994, V180, N1 (JUL 1), P223-231  
ISSN: 0022-1007  
Language: ENGLISH Document Type: ARTICLE

Abstract: We have previously shown that dendritic cells isolated after overnight culture, which can express B7 and are potent stimulators of naive T cell proliferation, are relatively poor at inducing the proliferation of a panel of murine T helper 1 (Th1) clones. Maximal stimulation of Th1 clones was achieved using unseparated splenic antigen presenting cells (APC). An explanation for these findings is provided in the present study where we show that FcR(+) L cells transfected with B7 stimulate minimal proliferation of Th1 clones in response to anti-CDS antibodies, in contrast to induction of significant proliferation of naive T cells. However, addition of interleukin 12 (IL-12) to cultures of Th1 cells stimulated with anti-CDS and FcR(+) B7 transfectants resulted in a very pronounced increase in proliferation and interferon gamma (IFN-gamma) production. Exogenous IL-12 did not affect the B7-induced proliferation of naive T cells. This showed that whereas costimulatory signals delivered via B7-CD28 interaction are sufficient to induce significant proliferation of naive T cells activated through occupancy of the **T cell receptor**, Th1 T cell clones require cooperative costimulation by B7 and IL-12. This costimulation was shown to be specific by inhibition of proliferation and IFN-gamma production using chimeric **soluble** cytolytic T lymphocyte-associated antigen 4-human IgG(1)Fc (CTLA4-Ig) and anti-IL-12 antibodies. Furthermore, the significant antigen specific proliferation and IFN-gamma production by Th1 clones observed when splenocytes were used as APC was almost completely abrogated using CTLA4-Ig and anti-IL-12 antibodies. Thus two costimulatory signals, B7 and IL-12, account for the ability of splenic APC to induce maximal stimulation of Th1 clones. IL-10 downregulates the expression of IL-12 by IFN-gamma-stimulated macrophages and this may account largely for the ability of IL-10 to inhibit APC function of splenic and macrophage APC for the induction of Th1 cell proliferation and IFN-gamma production. Indeed we show that IL-12 can overcome the inhibitory effect of IL-10 for the APC-dependent induction of proliferation and IFN-gamma production by Th1 clones. These results suggest that proliferation by terminally differentiated Th1 clones, in contrast to naive T cells, requires stimulation via membrane-bound B7 and a cytokine, IL-12. It is possible that these signals may result in the activation of unresponsive T cells during an inflammatory response. IL-10, by its role in regulating such innate inflammatory responses, may thus help to maintain these T cells in an unresponsive state.

...Abstract: are sufficient to induce significant proliferation of naive T cells activated through occupancy of the **T cell receptor**, Th1 T cell clones require cooperative costimulation by



B7 and IL-12. This costimulation was shown to be specific by inhibition of proliferation and IFN-gamma production using chimeric **soluble** cytolytic T lymphocyte-associated antigen 4-human IgG(1)Fc (CTLA4-Ig) and anti-IL...

...Identifiers--CELL STIMULATORY FACTOR; LYMPHOCYTE MATURATION FACTOR; NATURAL-KILLER-CELLS; DENDRITIC CELLS; MONOCLONAL-ANTIBODIES; **HETERODIMERIC** CYTOKINE; CD28 PATHWAY; TH1 CELLS; ANTIGEN; EXPRESSION

9/3,K,AB/30 (Item 12 from file: 34)  
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci  
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03189176 Genuine Article#: NM065 Number of References: 75  
Title: REGULATION OF THE AVIDITY OF INTEGRIN ALPHA(4)BETA(7) BY THE BETA(7) CYTOPLASMIC DOMAIN (Abstract Available)



4560212    Genuine Article#: TT376    Number of References: 28

Title: STRUCTURAL-ANALYSIS OF GAMMA-DELTA-TCR USING A NOVEL SET OF TCR  
GAMMA-CHAIN-SPECIFIC AND DELTA-CHAIN-SPECIFIC MONOCLONAL-ANTIBODIES  
GENERATED AGAINST **SOLUBLE** GAMMA-DELTA-TCR - EVIDENCE FOR A  
SPECIFIC CONFORMATION ADOPTED BY THE J-DELTA-2 REGION AND FOR A  
V-DELTA-1 POLYMORPHISM (Abstract Available)

Author(s): ROMAGNE F; PEYRAT MA; LEGET C; DAVODEAU F; HOUDE I; NECKER A;  
HALLET MM; VIE H; BONNEVILLE M

Corporate Source: INST BIOL,INSERM U211,9 QUAI MONCOUSTA/F-44035 NANTES  
01//FRANCE//; INST BIOL,INSERM U211/F-44035 NANTES 01//FRANCE//;  
IMMUNOTECH SA/F-13288 MARSEILLE//FRANCE/

Journal: JOURNAL OF IMMUNOLOGICAL METHODS, 1996, V189, N1 (JAN 16), P25-36  
ISSN: 0022-1759

Language: ENGLISH    Document Type: ARTICLE

Abstract: We recently showed that secretion of non-chimeric  
disulfide-linked human gamma delta TCR ('soluble' TCR, sTCR)  
comprising V gamma 9 and V delta 2 regions could be achieved by simply  
introducing translational termination codons upstream from the  
sequences encoding TCR transmembrane region. Here we extended these  
findings by demonstrating efficient secretion and  
**heterodimerization** of gamma delta sTCR comprising V gamma 8; V  
delta 1 and V delta 3 regions, obtained via the same strategy. After  
immunization against immunoaffinity-purified **soluble** TCR, several  
hundreds of TCR-specific monoclonal antibodies (mAb) were generated,  
which fell in at least seven groups. One set of mAb was directed  
against a V gamma 8-specific epitope. Strikingly, despite the high  
degree of sequence homology between V gamma 8 and other V gamma I  
domains, none of these mAb were crossreactive with other members of the  
V gamma I family. Three other sets of mAbs were shown to recognize  
delta chains comprising V delta 1, V delta 2 and V delta 3 regions  
respectively, regardless of their junctional sequence or of the gamma  
chain to which they were paired. Among the V delta 1-specific mAb, some  
specifically recognized V delta 1D delta J delta C delta chains while  
others reacted with both V delta 1 delta J delta C delta and V delta 1J  
alpha C alpha chains, which suggested V domain conformational  
alterations induced by the C region. Moreover, reactivity of one V  
delta 1-specific mAb (#R6.11) was affected by a polymorphic residue  
located on the predicted CDR4 loop of the V delta region. Two delta  
chain-specific mAb (#178 and #515) showed a highly unusual reactivity,  
which was negatively affected by particular V delta and J delta  
sequences: (i) mAb #515 and #178 recognized all TCR delta chains except  
those comprising V delta 1 or V delta 2 regions, respectively and (ii)  
within TCR delta chains carrying 'permissive' V delta regions, none of  
those comprising the J delta 2 region were recognized by #515 and/or  
#178 mAbs, which suggested a highly specific conformation adopted by  
this particular J delta sequence. Apart from its usefulness in TCR  
structural studies, this novel set of mAb represents an important tool  
for the characterization and isolation of gamma delta T cells  
expressing particular combinations of V gamma/V delta regions and for  
analysis of V alpha/V delta usage by alpha beta T cells. Moreover,  
since our present data strongly suggest that gamma delta TCR are easier  
to obtain in a **soluble** form than cup TCR, an efficient strategy  
for the generation of Ver region-specific mAb might be to immunize with  
chimeric gamma delta sTCR comprising particular V alpha regions.

...Title: NOVEL SET OF TCR GAMMA-CHAIN-SPECIFIC AND DELTA-CHAIN-SPECIFIC  
MONOCLONAL-ANTIBODIES GENERATED AGAINST **SOLUBLE** GAMMA-DELTA-TCR -  
EVIDENCE FOR A SPECIFIC CONFORMATION ADOPTED BY THE J-DELTA-2 REGION...

Abstract: We recently showed that secretion of non-chimeric  
disulfide-linked human gamma delta TCR ('soluble' TCR, sTCR)  
comprising V gamma 9 and V delta 2 regions could be achieved by...

...sequences encoding TCR transmembrane region. Here we extended these  
findings by demonstrating efficient secretion and



heterodimerization of gamma delta sTCR comprising V gamma 8; V delta 1 and V delta 3 regions, obtained via the same strategy. After immunization against immunoaffinity-purified **soluble** TCR, several hundreds of TCR-specific monoclonal antibodies (mAb) were generated, which fell in at...

...our present data strongly suggest that gamma delta TCR ar



5408295    Genuine Article#: VX026    Number of References: 48

Title: ASSEMBLY, SPECIFIC BINDING, AND CRYSTALLIZATION OF A HUMAN  
TCR-ALPHA-BETA WITH AN ANTIGENIC TAX PEPTIDE FROM HUMAN T-LYMPHOTROPIC  
VIRUS TYPE-1 AND THE CLASS-I MHC MOLECULE HLA-A2 (Abstract Available)

Author(s): GARBOCZI DN; UTZ U; GHOSH P; SETH A; KIM J; VANTIENHOVEN EAE;  
BIDDISON WE; WILEY DC

Corporate Source: HARVARD UNIV,DEPT MOL & CELLULAR BIOL,7 DIVIN  
AVE/CAMBRIDGE//MA/02138; HARVARD UNIV,DEPT MOL & CELLULAR  
BIOL/CAMBRIDGE//MA/02138; HARVARD UNIV,HOWARD HUGHES MED  
INST/CAMBRIDGE//MA/02138; CLIN RES INST MONTREAL,IMMUNOL  
LAB/MONTREAL/PQ H2W 1R7/CANADA/; NINCDS,MOL IMMUNOL SECT,NEUROIMMUNOL  
BRANCH,NIH/BETHESDA//MD/20892; CHILDRENS HOSP,HOWARD HUGHES MED  
INST,MOL MED LAB/BOSTON//MA/02115

Journal: JOURNAL OF IMMUNOLOGY, 1996, V157, N12 (DEC 15), P5403-5410

ISSN: 0022-1767

Language: ENGLISH    Document Type: ARTICLE

Abstract: T lymphocytes use TCR-alpha beta to bind and to recognize  
complexes of antigenic peptides bound to MHC proteins located at the  
surface of APCs, We have assembled and crystallized this intercellular  
complex of TCR/peptide/MHC from **soluble** human TCR-alpha beta and  
**soluble** peptide/HLA-A2 complexes. The **soluble** TCR-alpha  
beta binds specifically to its in vivo ligand, the complex of HLA-A2,  
and a peptide from the Tax protein of human T lymphotropic virus type  
1. The **soluble** TCR also binds in vitro to an altered peptide  
ligand, which appears to be a partial agonist in T cell assays as  
determined by its ability to elicit different cytolytic and lymphokine  
secretion responses, **Heterodimerization** and the antigenic  
specificity of the TCR do not require its interchain disulfide bond,  
transmembrane segments, or glycosylations, Crystals of the  
TCR/peptide/HLA-A2 complex diffract x-rays, providing the means to  
study in atomic detail the mechanism of Ag-specific cell-cell  
recognition between T cells and target cells.



9/3,K,AB/18 (Item 6 from file: 55)  
DIALOG(R)File 55:Biosis Previews(R)  
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0009292841 BIOSIS NO.: 199497314126

Unique antigen recognition by a herpesvirus-specific TCR-gamma-delta cell  
AUTHOR: Sciammas Roger (Reprint); Johnson Raymond M; Sperling Anne I; Brady  
William; Linsley Peter S; Spear Patricia G; Fitch Frank W; Bluestone  
Jeffrey A

AUTHOR ADDRESS: Ben May Inst. and Committee on Immunol., Univ. Chicago,  
MC1089, 5841 S. Maryland Ave., Chicago, IL 60637, USA\*\*USA

JOURNAL: Journal of Immunology 152 (11): p5392-5397 1994 1994

ISSN: 0022-1767

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: TCR-gamma-delta cells, a T cell subset present in the epithelial and lymphoid tissues, have been implicated in viral and bacterial infections. We have identified a TCR-gamma-delta clone (Tg14.4) that, unlike TCR-alpha-beta cells, recognizes a herpes simplex virus type 1 transmembrane glycoprotein, gl, in an MHC class I- and class II-independent fashion. The TCR of Tg14.4 is composed of rearranged V-delta-8 (a V-alpha-2 family member) and V-gamma-1.2 variable genes, a **heterodimeric** pair not previously described. Furthermore, anti-V-alpha-2 mAbs are sufficient to block recognition of the gl ligand. Strikingly, anti-gl Abs also are capable of blocking recognition, a phenomena that is very rare in TCR-alpha-beta Ag recognition. Therefore, to dissect the mechanism involved in this unique form of Ag recognition, we constructed a mutant of gl, glt, that lacks cell surface expression upon transfection into APCs. This form of gl was not sufficient for Ag presentation. In contrast, wild-type gl expressed in the Ag-processing mutant cell, RMA-S, is recognized by Tg14.4, suggesting that gl presentation occurs independently of classical Ag-processing pathways. In fact, through the use of a **soluble** recombinant gl molecule, gl-lg, we show that Tg14.4 can recognize whole, unprocessed gl protein in the absence of any APCs. These results suggest that there exist alternate and novel forms of TCR Ag recognition, and that the TCR-gamma-delta clone, Tg14.4, may represent a novel T cell subset that, during pathogenic challenge, may respond directly to Ags on the surfaces of bacteria and viruses.

...ABSTRACT: 8 (a V-alpha-2 family member) and V-gamma-1.2 variable genes, a **heterodimeric** pair not previously described. Furthermore, anti-V-alpha-2 mAbs are sufficient to block recognition...

...presentation occurs independently of classical Ag-processing pathways. In fact, through the use of a **soluble** recombinant gl molecule, gl-lg, we show that Tg14.4 can recognize whole, unprocessed gl...

DESCRIPTORS:

MISCELLANEOUS TERMS: ...T CELL RECEPTOR;

9/3,K,AB/19 (Item 1 from file: 34)  
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci  
(c) 2004 Inst for Sci Info. All rts. reserv.

10307298 Genuine Article#: 511PM Number of References: 90

Title: Adoptive T-cell therapy for the treatment of solid tumours (

ABSTRACT AVAILABLE)

Author(s): Knutson KL (REPRINT) ; Almand B; Mankoff DA; Schiffman K; Disis  
ML

Corporate Source: Univ Washington, Div Oncol, 1959 NE Pacif St, HSB BB1321, Box  
356527/Seattle//WA/98195 (REPRINT); Univ Washington, Div



Oncol,Seattle//WA/98195; Univ Washington,Div Nucl Med,Seattle//WA/98195  
; Univ Washington,Div Oncol,Seattle//WA/98195  
Journal: EXPERT OPINION ON BIOLOGICAL THERAPY, 2002, V2, N1 (JAN), P55-66  
ISSN: 1471-2598 Publication date: 20020100  
Publisher: ASHLEY PUBLICATIONS LTD, UNITEC HOUSE, 3RD FL, 2 ALBERT PLACE  
FINCHLEY CENTRAL, LONDON N3 1QB, ENGLAND  
Language: English Document Type: REVIEW



0009399393 BIOSIS NO.: 199497420678

Peripheral T cell receptors alpha-beta and gamma-delta in patients with  
Hodgkin's lymphoma

AUTHOR: Akan Hamdi (Reprint); Beksac Meral; Aydogdu Ismet; Koc Haluk; Ilhan  
Osman; Ozcan Muhit

AUTHOR ADDRESS: Ugur Mumcu Sok. 53/7, Buyukesat 06700, Ankara, Turkey\*\*  
Turkey

JOURNAL: British Journal of Haematology 87 (3): p544-547 1994 1994

ISSN: 0007-1048

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Antigen recognition by T cells is determined by an antigen  
specific **T cell receptor** (TCR). Two **heterodimeric**

TCR structures associated with CD3 have been defined: TCR alpha-beta and  
TCR gamma-delta. TCR alpha-beta and its function are well described but  
the role of TCR gamma-delta in normal and lymphoproliferative disorders  
is not well established. In newly diagnosed or relapsed/refractory  
Hodgkin's disease (HD), a disease associated with defective T cell  
functions and increased sIL-2R, we determined levels of seven TCR  
alpha-beta variable regions (beta-V5(a), beta-V5(b), beta-v6(a),  
beta-V12(a), alpha-beta-V(a), alpha-V2(a)) and TCR beta-delta by using  
monoclonal antibodies (MCA). TCR gamma-delta levels did not show any  
difference, but several variable regions of the TCR alpha-beta differed  
when groups are compared with each other and the control group.



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0013075124 BIOSIS NO.: 200100246963

Polynucleotides encoding molecular complexes which modify immune responses

AUTHOR: Schneck Jonathan; O'Herrin Sean; Lebowitz Michael S (Reprint);

Hamad Abdel

AUTHOR ADDRESS: Pikesville, MD, USA\*\*USA

JOURNAL: Official Gazette of the United States Patent and Trademark Office

Patents 1239 (5): Oct. 31, 2000 2000

MEDIUM: e-file

PATENT NUMBER: US 6140113 PATENT DATE GRANTED: October 31, 2000 20001031

PATENT CLASSIFICATION: 435-3201 PATENT ASSIGNEE: The Johns Hopkins

University PATENT COUNTRY: USA

ISSN: 0098-1133

DOCUMENT TYPE: Patent

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Polynucleotides encode **soluble**, multivalent molecular complexes which modify immune responses, and host cells comprise such polynucleotides. The molecular complexes comprise extracellular domains of transmembrane **heterodimeric** proteins, particularly **T cell receptor** and major histocompatibility complex proteins, which are covalently linked to the heavy and light chains of immunoglobulin molecules to provide **soluble** multivalent molecular complexes with high affinity for their cognate ligands. The molecular complexes can be used, inter alia, to detect and regulate antigen-specific T cells and as therapeutic agents for treating disorders involving immune system regulation, such as allergies, autoimmune diseases, tumors, infections, and transplant rejection.



0014016072 BIOSIS NO.: 200200609583

Molecular complexes which modify immune responses

AUTHOR: Schneck Jonathan; O'Herrin Sean (Reprint); Lebowitz Michael S;  
Hamad Abdel

AUTHOR ADDRESS: Madison, WI, USA\*\*USA

JOURNAL: Official Gazette of the United States Patent and Trademark Office  
Patents 1263 (1): Oct. 1, 2002 2002

MEDIUM: e-file

PATENT NUMBER: US 6458354 PATENT DATE GRANTED: October 01, 2002 20021001

PATENT CLASSIFICATION: 424-1341 PATENT ASSIGNEE: The Johns Hopkins

University PATENT COUNTRY: USA

ISSN: 0098-1133

DOCUMENT TYPE: Patent

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Extracellular domains of transmembrane **heterodimeric** proteins, particularly **T cell receptor** and major histocompatibility complex proteins, can be covalently linked to the heavy and light chains of immunoglobulin molecules to provide **soluble** multivalent molecular complexes with high affinity for their cognate ligands. The molecular complexes can be used, inter alia, to detect and regulate antigen-specific T cells and as therapeutic agents for treating disorders involving immune system regulation, such as allergies, autoimmune diseases, tumors, infections, and transplant rejection.



9/3,K,AB/10 (Item 10 from file: 155)  
DIALOG(R) File 155:MEDLINE(R)  
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06882040 91122149 PMID: 1671358

**Heterodimeric**, disulfide-linked alpha/beta T cell receptors in solution.

Slanetz A E; Bothwell A L

Department of Biology, Yale University School of Medicine, New Haven, CT 06510.

European journal of immunology (GERMANY) Jan 1991, 21 (1) p179-83,  
ISSN 0014-2980 Journal Code: 1273201

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Structural and functional analysis of **T cell receptor** (TcR)-ligand binding would be greatly advanced by the availability of an intact, assembled TcR in **soluble** form. We have produced such a molecule, by splicing the extracellular domains of a TcR to the glycosyl phosphatidylinositol membrane anchor sequences of Thy-1. The molecule is expressed in the absence of CD3 on the cell surface, and can be cleaved from the membrane by treatment with phosphatidylinositol-specific phospholipase C. The alpha and beta chains of the **soluble** molecule are paired in the native conformation as judged by reactivity with the anti-V beta 8 monoclonal antibody F23.1, and with the anti-clonotypic monoclonal antibody 1B2; it is a disulfide-linked dimer with a mol. mass of 95 kDa on sodium dodecyl sulfate-polyacrylamide gel electrophoresis under nonreducing conditions, and 47 kDa after reduction. We conclude that we have generated an alpha/beta TcR in **soluble** form.



779151 20060993 PMID: 10595544

Production of **soluble** alphabeta T-cell receptor heterodimers suitable for biophysical analysis of ligand binding.

Willcox B E; Gao G F; Wyer J R; O'Callaghan C A; Boulter J M; Jones E Y; van der Merwe P A; Bell J I; Jakobsen B K

MRC Human Immunology Unit, Institute of Molecular Medicine, John Radcliffe Hospital, Oxford, United Kingdom.

Protein science - a publication of the Protein Society (UNITED STATES)  
Nov 1999, 8 (11) p2418-23, ISSN 0961-8368 Journal Code: 9211750

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

A method to produce alphabeta T-cell receptors (TCRs) in a **soluble** form suitable for biophysical analysis was devised involving in vitro refolding of a TCR fusion protein. Polypeptides corresponding to the variable and constant domains of each chain of a human and a murine receptor, fused to a coiled coil **heterodimerization** motif from either c-Jun (alpha) or v-Fos (beta), were overexpressed separately in *Escherichia coli*. Following recovery from inclusion bodies, the two chains of each receptor were denatured, and then refolded together in the presence of denaturants. For the human receptor, which is specific for the immunodominant influenza A HLA-A2-restricted matrix epitope (M58-66), a **heterodimeric** protein was purified in milligram yields and found to be homogeneous, monomeric, antibody-reactive, and stable at concentrations lower than 1 microM. Using similar procedures, analogous results were obtained with a murine receptor specific for an influenza nucleoprotein epitope (366-374) restricted by H2-Db. Production of these receptors has facilitated a detailed analysis of viral peptide-Major Histocompatibility Complex (peptide-MHC) engagement by the TCR using both surface plasmon resonance (SPR) and, in the case of the human TCR, isothermal titration calorimetry (ITC) (Willcox et al., 1999)<sup>o</sup>. The recombinant methods described should enable a wide range of TCR-peptide-MHC interactions to be studied and may also have implications for the production of other **heterodimeric** receptor molecules.



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09149158 20451136 PMID: 10993731

**Heterodimeric** CD3epsilon gamma extracellular domain fragments: production, purification and structural analysis.

Kim K S; Sun Z Y; Wagner G; Reinherz E L

Laboratory of Immunobiology, Dana-Farber Cancer Institute and Department of Medicine, Boston, MA, 02115, USA.

Journal of molecular biology (ENGLAND) Sep 29 2000, 302 (4) p899-916

, ISSN 0022-2836 Journal Code: 2985088R

Contract/Grant No.: AI19807; AI; NIAID; AI37581; AI; NIAID

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The CD3 polypeptides (epsilon, gamma, and delta) are non-covalently associated signaling subunits of the **T cell receptor** which form non-disulfide linked epsilon gamma and epsilon delta heterodimers. With the goal of investigating their structure, Escherichia coli expression was utilized to produce CD3 ectodomain fragments including the murine CD3epsilon subunit N-terminal Ig-like extracellular domain alone or as a single chain construct with that of CD3gamma. The latter links the CD3gamma segment to the C terminus of the CD3epsilon segment via a 26 amino acid peptide (scCD3epsilon gamma26). Although CD3epsilon could be produced at high yield when directed to inclusion bodies, the refolded monomeric CD3epsilon was not native as judged by monoclonal antibody binding using surface plasmon resonance and was largely unstructured by (15)N-(1)H two-dimensional NMR analysis. In contrast, scCD3epsilon gamma26 could be refolded readily into a native state as shown by CD, NMR and mAb reactivity. The linker length between CD3epsilon and CD3gamma is critical since scCD3epsilon gamma16 containing a 16 residue connector failed to generate a stable heterodimer. Collectively, the results demonstrate that: (i) **soluble heterodimeric** fragments of CD3 can be produced; (ii) cotranslation of CD3 chains insures proper folding even in the absence of the conserved ectodomain stalk region (CxxCxE); and (iii) CD3epsilon has a more stable tertiary protein fold than CD3gamma. Copyright 2000 Academic Press.

**Heterodimeric** CD3epsilon gamma extracellular domain fragments: production, purification and structural analysis.

The CD3 polypeptides (epsilon, gamma, and delta) are non-covalently associated signaling subunits of the **T cell receptor** which form non-disulfide linked epsilon gamma and epsilon delta heterodimers. With the goal of investigating their...

... 16 residue connector failed to generate a stable heterodimer. Collectively, the results demonstrate that: (i) **soluble heterodimeric** fragments of CD3 can be produced; (ii) cotranslation of CD3 chains insures proper folding even...

9/3,K,AB/7 (Item 7 from file: 155)

DIALOG(R) File 155:MEDLINE(R)

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08779151 20060993 PMID: 10595544

Production of **soluble** alphabeta **T-cell receptor**

heterodimers suitable for biophysical analysis of ligand binding.

Willcox B E; Gao G F; Wyer J R; O'Callaghan C A; Boulter J M; Jones E Y; van der Merwe P A; Bell J I; Jakobsen B K

MRC Human Immunology Unit, Institute of Molecular Medicine, John Radcliffe Hospital, Oxford, United Kingdom.

Protein science - a publication of the Protein Society (UNITED STATES)  
Nov 1999, 8 (11) p2418-23, ISSN 0961-8368 Journal Code: 9211750

Document type: Journal Article



Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

A method to produce alphabeta T-cell receptors (TCRs) in a **soluble** form suitable for biophysical analysis was devised involving in vitro refolding of a TCR fusion protein. Polypeptides corresponding to the variable and constant domains of each chain of a human and a murine receptor, fused to a coiled coil **heterodimerization** motif from either c-Jun (alpha) or v-Fos (beta), were overexpressed separately in Escherichia coli. Following recovery from inclusion bodies, the two chains of each receptor were denatured, and then refolded together in the presence of denaturants. For the human receptor, which is specific for the immunodominant influenza A HLA-A2-restricted matrix epitope (M58-66), a **heterodimeric** protein was purified in milligram yields and found to be homogeneous, monomeric, antibody-reactive, and stable at concentrations lower than 1 microM. Using similar procedures, analogous results were obtained with a murine receptor specific for an influenza nucleoprotein epitope (366-374) restricted by H2-Db. Production of these receptors has facilitated a detailed analysis of viral peptide-Major Histocompatibility Complex (peptide-MHC) engagement by the TCR using both surface plasmon resonance (SPR) and, in the case of the human TCR, isothermal titration calorimetry (ITC) (Willcox et al., 1999). The recombinant methods described should enable a wide range of TCR-peptide-MHC interactions to be studied and may also have implications for the production of other **heterodimeric** receptor molecules.

Production of **soluble** alphabeta T-cell receptor heterodimers suitable for biophysical analysis of ligand binding.

A method to produce alphabeta T-cell receptors (TCRs) in a **soluble** form suitable for biophysical analysis was devised involving in vitro refolding of a TCR fusion...

...of each chain of a human and a murine receptor, fused to a coiled coil **heterodimerization** motif from either c-Jun (alpha) or v-Fos (beta), were overexpressed separately in Escherichia...

... is specific for the immunodominant influenza A HLA-A2-restricted matrix epitope (M58-66), a **heterodimeric** protein was purified in milligram yields and found to be homogeneous, monomeric, antibody-reactive, and...

... MHC interactions to be studied and may also have implications for the production of other **heterodimeric** receptor molecules.

9/3,K,AB/8 (Item 8 from file: 155)

DIALOG(R) File 155:MEDLINE(R)

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08597414 95285731 PMID: 7768153

Primitive vertebrate immunity: what is the evolutionary derivative of molecules that define the adaptive immune system?

Flajnik M F

Department of Microbiology and Immunology, University of Miami, FL 33136, USA.

Ciba Foundation symposium (NETHERLANDS) 1994, 186 p224-32; discussion 233-6, ISSN 0300-5208 Journal Code: 0356636

Contract/Grant No.: AI27877; AI; NIAID; RR06603; RR; NCRR

Document type: Journal Article; Review; Review, Tutorial

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The adaptive immune system is capable of responding to an infinite number of antigens with the antigen-specific receptors immunoglobulin (Ig) and the **T cell receptor** (TCR). Ig binds **soluble** antigens



while TCR recognizes antigen bound in clefts of polymorphic self-encoded major histocompatibility complex (MHC) class I and class II



14976739 22340338 PMID: 12454477

The production, purification and crystallization of a **soluble heterodimeric** form of a highly selected **T-cell receptor** in its unliganded and liganded state.

Clements Craig S; Kjer-Nielsen Lars; MacDonald Whitney A; Brooks Andrew G ; Purcell Anthony W; McCluskey James; Rossjohn Jamie

The Protein Crystallography Unit, Department of Biochemistry and Molecular Biology, School of Biomedical Sciences, Monash University, Clayton, Victoria 3168, Australia.

Acta crystallographica. Section D, Biological crystallography (Denmark)

Dec 2002, 58 (Pt 12) p2131-4, ISSN 0907-4449 Journal Code: 9305878

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

T-cell antigen receptors (TCRs) are **heterodimeric** cell-surface receptors that play a pivotal role in the cellular immune response. The TcR interacts specifically with a peptide-laden major histocompatibility complex (pMHC). A human TcR has been characterized that interacts with an immunodominant epitope, FLRGRAYGL, from the Epstein-Barr virus, a ubiquitous human pathogen, in complex with HLA-B8. Despite the vast TcR repertoire, this TcR is found in up to 10% of the total T-cell population in seropositive HLA-B8+ individuals. In this report, this highly selected TcR is characterized by expressing in Escherichia coli, refolding, purifying and crystallizing the receptor. In addition, the HLA-B8-FLRGRAYGL complex has been expressed in E. coli, refolded and shown to be functionally active. Using native gel electrophoresis, the refolded TcR is shown to be capable of binding specifically to the refolded HLA-B8-FLRGRAYGL and this TcR has been crystallized in complex with the pMHC. The crystals of the unliganded and liganded TcR diffract to 1.5 and 2.5 Å, respectively.

The production, purification and crystallization of a **soluble heterodimeric** form of a highly selected **T-cell receptor** in its unliganded and liganded state.



9/3,K,AB/40 (Item 8 from file: 340)  
DIALOG(R) File 340:CLAIMS(R)/US Patent  
(c) 2004 IFI/CLAIMS(R). All rts. reserv.

Dialog Acc No: 2948062 IFI Acc No: 9806179  
Document Type: C  
PRODUCTION OF SUBUNITS OF **SOLUBLE** T CELL RECEPTORS BY CO-TRANSFECTION  
Inventors: Bonneville Marc (FR)  
Assignee: Immunotech FR; Institut National de la Sante et de la Recherche  
Medicale FR  
Assignee Code: 16381 42342  
Publication (No,Date), Applic (No,Date):  
US 5723309 19980303 US 94256964 19940914  
Publication Kind: A  
Calculated Expiration: 20150303  
PCT Pub(No,Date),Applic(No,Date): WO 9412648 19940609 WO 93FR1165  
19931125  
Section 371: 19940914  
Section 102(e):19940914  
Priority Applic(No,Date): FR 9214203 19921125

Abstract: **Soluble**, single chain T cell receptors, nucleic acid sequences, particularly DNA sequences, encoding the **soluble**, single chain **T cell receptor**, expression vectors containing the DNA sequences, and host cells containing the expression vectors.

#### PRODUCTION OF SUBUNITS OF **SOLUBLE** T CELL RECEPTORS BY CO-TRANSFECTION

Abstract: **Soluble**, single chain T cell receptors, nucleic acid sequences, particularly DNA sequences, encoding the **soluble**, single chain **T cell receptor**, expression vectors containing the DNA sequences, and host cells containing the expression vectors.

Exemplary Claim: D R A W I N G

1. Process for producing **soluble** T receptors, comprising cotransfecting into a host cell DNA sequences each encoding only a single...

Non-exemplary Claims: 2. Process according to claim 1, wherein V Alpha C/60 /V Beta C Beta **soluble** T receptors are produced by co-transfecting, into a host cell, said DNA sequences each...

...3. Process according to claim 1, wherein V gamma C gamma /V delta C delta **soluble** T receptors are produced by co-transfecting, into a host cell, said DNA sequences each...

...4. Process according to claim 1, wherein V Alpha C/65 /V Beta C delta **heterodimeric soluble** T receptors are produced, in which the constituent subunits are associated via a covalent bond...

...5. Process according to claim 1, wherein V Alpha C delta /V Beta C/65 **heterodimeric soluble** T receptors are produced, in which the constituent subunits are associated via a covalent bond...

...Process according to claim 1, wherein V gamma C gamma /V Alpha C delta hybrid **soluble** T receptors are produced by co-transfecting, into a host cell, a said DNA sequence...

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28jan04 14:01:51 User231882 Session D1264.3  
\$4.19 1.309 DialUnits File155  
\$3.15 15 Type(s) in Format 4 (UDF)  
\$3.15 15 Types



\$7.34 Estimated cost File155  
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        \$15.60 8 Type(s) in Format 4 (UDF)  
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    OneSearch, 5 files, 3.064 DialUnits FileOS  
    \$1.40 TELNET  
\$156.09 Estimated cost this search  
\$156.15 Estimated total session cost 3.286 DialUnits  
Logoff: level 03.07.00 D 14:01:51



Dialog Acc No: 3343591 IFI Acc No: 0019752

Document Type: C

**SOLUBLE T CELL RECEPTORS; MEMBRANE-FREE HETERODIMERIC,  
DISULFIDE LINKED ALPHA/BETA SOLUBLE T CELL RECEPTOR**  
SHARING A CLONOTYPIC EPITOPE WITH A NATIVE RECEPTOR; FOR SCREENING LIGANDS;  
FOR IMMUNOSUPPRESSANTS AND THERAPY FOR AUTOIMMUNE DISEASES

Inventors: Bothwell Alfred L M (US); Slanetz Alfred E (US)

Assignee: Unassigned Or Assigned To Individual

Assignee Code: 68000

Publication (No,Date), Applic (No,Date):

US 6080840 20000627 US 95393157 19950217

Publication Kind: A

Calculated Expiration: 20170627

Continuation Pub(No),Applic(No,Date): ABANDONED

US 92822538

19920117

Cont.-in-part Pub(No),Applic(No,Date): ABANDONED

US

93168782 19931214

Priority Applic(No,Date): US 95393157 19950217; US 92822538 19920117;

US 93168782 19931214

Abstract: An intact, assembled **T cell receptor** (TcR) in **soluble** form is provided. The **soluble** TcR is prepared by splicing the extracellular domains of a **T cell receptor** to the glycosyl phosphatidylinositol (GPI) membrane anchor sequences of Thy-1. The molecule is expressed in the absence of CD3 on the cell surface, and can be cleaved from the membrane by treatment with phosphatidylinositol specific phospholipase C (PI-PLC). The alpha and beta chains of the **soluble** molecule are paired in the native conformation as judged by reactivity with the anti-V beta 8 monoclonal antibody F23.1, and with the anti-clonotypic monoclonal antibody 1B2. The **soluble** TcR is a disulfide linked dimer with a molecular mass of 95 kDa on SDS-PAGE under nonreducing conditions, and 47 kDa after reduction.

**SOLUBLE T CELL RECEPTORS...**



Dialog Acc No: 3409072 IFI Acc No: 0036169

Document Type: C

POLYNUCLEOTIDES ENCODING MOLECULAR COMPLEXES WHICH MODIFY IMMUNE RESPONSES;  
NUCLEOTIDE SEQUENCES WHICH CODES AN IMMUNOGLOBULIN FUSION PROTEIN; FOR THE  
DETECTION AND TREATMENT OF ALLERGIES, AUTOIMMUNE DISEASES, TUMORS,  
INFECTIONS, AND TRANSPLANT EJECTION; ANTITUMOR AGENTS

Inventors: Hamad Abdel (US); Lebowitz Michael S (US); O'Herrin Sean (US);  
Schneck Jonathan (US)

Assignee: Johns Hopkins University

Assignee Code: 39884

Publication (No,Date), Applic (No,Date):

US 6140113 20001031 US 9863276 19980421

Publication Kind: A

Calculated Expiration: 20170328

Cont.-in-part Pub(No), Applic(No,Date): US 6015884 US

97828712 19970328

Priority Applic(No,Date): US 9863276 19980421; US 97828712 19970328

Abstract: Polynucleotides encode **soluble**, multivalent molecular complexes which modify immune responses, and host cells comprise such polynucleotides. The molecular complexes comprise extracellular domains of transmembrane **heterodimeric** proteins, particularly **T cell receptor** and major histocompatibility complex proteins, which are covalently linked to the heavy and light chains of immunoglobulin molecules to provide **soluble** multivalent molecular complexes with high affinity for their cognate ligands. The molecular complexes can be used, inter alia, to detect and regulate antigen-specific T cells and as therapeutic agents for treating disorders involving immune system regulation, such as allergies, autoimmune diseases, tumors, infections, and transplant rejection.



Dialog Acc No: 3760508 IFI Acc No: 0235137

Document Type: C

MOLECULAR COMPLEXES WHICH MODIFY IMMUNE RESPONSES

Inventors: Hamad Abdel (US); Lebowitz Michael S (US); O'Herrin Sean (US);  
Schneck Jonathan (US)

Assignee: Johns Hopkins University

Assignee Code: 39884

Publication (No,Date), Applic (No,Date):

US 6458354 20021001 US 2000668143 20000925

Publication Kind: B

Calculated Expiration: 20170328

Cont.-in-part Pub(No),Applic(No,Date): Pending

97828712 19970328; US 6140113 US 9863276 US 19980421;

Pending US 99324782 19990603

Priority Applic(No,Date): US 2000668143 20000925; US 97828712 19970328;

US 9863276 19980421; US 99324782 19990603

Abstract: Extracellular domains of transmembrane **heterodimeric** proteins, particularly **T cell receptor** and major histocompatibility complex proteins, can be covalently linked to the heavy and light chains of immunoglobulin molecules to provide **soluble** multivalent molecular complexes with high affinity for their cognate ligands. The molecular complexes can be used, inter alia, to detect and regulate antigen-specific T cells and as therapeutic agents for treating disorders involving immune system regulation, such as allergies, autoimmune diseases, tumors, infections, and transplant rejection.

Abstract: Extracellular domains of transmembrane **heterodimeric** proteins, particularly **T cell receptor** and major histocompatibility complex proteins, can be covalently linked to the heavy and light chains of immunoglobulin molecules to provide **solu**



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File 155:MEDLINE(R) 1966-2004/Jan W3

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\*File 155: Medline is updating again (12-22-2003).

Please see HELP NEWS 154, for details.

File 55:Biosis Previews(R) 1993-2004/Jan W3

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File 34:SciSearch(R) Cited Ref Sci 1990-2004/Jan W3

(c) 2004 Inst for Sci Info

\*File 34: New prices as of 1/1/2004 per Information Provider request. See HELP RATES 34.

File 434:SciSearch(R) Cited Ref Sci 1974-1989/Dec

(c) 1998 Inst for Sci Info

\*File 434: New prices as of 1/1/2004 per Information Provider request. See HELP RATES434.

File 340:CLAIMS(R)/US Patent 1950-04/Jan 22

(c) 2004 IFI/CLAIMS(R)

\*File 340: Annual reload and classification updates delayed due to processing issues.

Set Items Description

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? s t(w)cell(w)receptor

Processing

4717141 T

4976899 CELL

1635074 RECEPTOR

S1 72541 T(W)CELL(W)RECEPTOR

? s extracellular

S2 409838 EXTRACELLULAR

? s s1 and s2

72541 S1

409838 S2

S3 2225 S1 AND S2

? s disulfide

S4 75966 DISULFIDE

? s s3 and s4

2225 S3

75966 S4

S5 75 S3 AND S4

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>>>Records from unsupported files will be retained in the RD set.

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...completed examining records

S6 49 RD (unique items)

? s unstable

S7 119752 UNSTABLE

? s s6 and s7

49 S6

119752 S7

S8 0 S6 AND S7

? s fold?

S9 715867 FOLD?

? s s6 and s9

49 S6

715867 S9

S10 12 S6 AND S9

? t s10/3,k,ab/1-12

10/3,K,AB/1 (Item 1 from file: 155)

DIALOG(R)File 155:MEDLINE(R)



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11705318 99141420 PMID: 9917415

**Folding**, heterodimeric association and specific peptide recognition  
of a murine alphabeta **T-cell receptor** expressed in  
Escherichia coli.

Pecorari F; Tissot A C; Pluckthun A  
Biochemisches Institut, Universitat Zurich, Winterthurerstr. 190, Zurich,  
CH-8057, Switzerland.

Journal of molecular biology (ENGLAND) Jan 29 1999, 285 (4) p1831-43  
, ISSN 0022-2836 Journal Code: 2985088R  
Document type: Journal Article



03033194 Genuine Article#: MX149 Number of References: 50

Title: T-CELL ANTIGEN RECEPTOR EXPRESSION STUDIED USING SOMATIC-CELL  
MUTANTS (Abstract Available)

Author(s): CASPARBAUGUIL S; ARNAUD J; RUBIN B

Corporate Source: CHU PURPAN, CRPG, IMMUNOL CELLULAIRE & MOLEC

LAB, CNRS/F-31300 TOULOUSE//FRANCE/; CHU PURPAN, CRPG, IMMUNOL CELLULAIRE  
& MOLEC LAB, CNRS/F-31300 TOULOUSE//FRANCE/

Journal: COMPTES RENDUS DE L'ACADEMIE DES SCIENCES SERIE III-SCIENCES DE LA  
VIE-LIFE SCIENCES, 1994, V317, N1 (JAN), P77-84

ISSN: 0764-4469

Language: FRENCH Document Type: ARTICLE

Abstract: T lymphocytes express membrane antigen receptor TcR/CD3 complexes only when all subunits are correctly assembled. Studies on TcR/CD3 membrane negative T cell variants containing all necessary subunits intracellularly, may allow to identify amino acid important for different subunit interactions. In this review, we summarize our recent work on TcR/CD3 negative variants of the human T cell line Jurkat. We found two critical amino acid in the TcR-alpha and TcR-beta **extracellular** constant regions (phenylalanine n degrees 216 and intrachain **disulfide** cysteine n degrees 212) involved in TcR-alpha beta/CD3-gamma epsilon, delta epsilon intermediary complex/zeta(2) homodimer interactions: (1) amino acid exchanges of phenylalanine demonstrated the importance of an aromatic amino acid residue at this position; (2) the intrachain **disulfide** bond assures a tertiary structure of the constant domain that is necessary for association with zeta(2) homodimers.

...Abstract: line Jurkat. We found two critical a



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04567474 Genuine Article#: TU126 Number of References: 19

Title: CONFORMATION OF THE T-CELL ANTIGEN RECEPTOR-BETA CHAIN C-DOMAIN  
CONTRIBUTES TO V-BETA(3) EPITOPE RECOGNITION BY MONOCLONAL-ANTIBODY  
KJ25 (Abstract Available)

Author(s): LI ZG; KEMP O; LONGHURST T; MANOLIOS N

Corporate Source: ROYAL N SHORE HOSP,DEPT RHEUMATOL/SYDNEY/NSW

2065/AUSTRALIA/; ROYAL N SHORE HOSP,DEPT RHEUMATOL/SYDNEY/NSW

2065/AUSTRALIA/; UNIV SYDNEY,DEPT MED/SYDNEY/NSW 2065/AUSTRALIA/; UNIV

TECHNOL SYDNEY,DEPT MOLEC & CELL BIOL,IMMUNOL UNIT/SYDNEY/NSW

2007/AUSTRALIA/

Journal: SCANDINAVIAN JOURNAL OF IMMUNOLOGY, 1996, V43, N2 (FEB), P140-145

ISSN: 0300-9475

Language: ENGLISH Document Type: ARTICLE

Abstract: The clonotypic T-cell antigen receptor (TCR)-beta chain contains two **extracellular** intrachain **disulfide** bonds. It belongs to the immunoglobulin gene superfamily and is subdivided into variable (V), joining (J), diversity (D) and constant (C) region. Monoclonal antibody (MoAb) KJ25 is believed to recognize an epitope in the V-domain of TCR-beta (V beta(3)) chain, but its epitope requirements are unknown. In this study of TCR-alpha beta chain interactions using chimeric recombinant TCR-beta chains, the authors found that partial substitution of the C beta-domain with that of interleukin-2 receptor alpha chain (Tac) sequences led to the loss of TCR-V beta(3) epitope recognition by KJ25. These results suggest that epitope recognition of the TCR-V beta(3) by KJ25 MoAb is dependent not only on the V-domain, but also on the close contact with the **extracellular** C-domain which influences the conformation and epitope recognition of the V beta(3)-region. This may not be unique to V beta(3) and may be a general feature of TCR-beta protein **folding**.

Abstract: The clonotypic T-cell antigen receptor (TCR)-beta chain contains two **extracellular** intrachain **disulfide** bonds. It belongs to the immunoglobulin gene superfamily and is subdivided into variable (V), joining...



8330262 95018232 PMID: 7932722

Correctly **folded T-cell receptor** fragments in the periplasm of Escherichia coli. Influence of **folding** catalysts.

Wulfig C; Pluckthun A

Max-Planck-Institut für Biochemie, Protein Engineering Group, Martinsried, Germany.

Journal of molecular biology (ENGLAND) Oct 7 1994, 242 (5) p655-69, ISSN 0022-2836 Journal Code: 2985088R

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The **T-cell receptor** is the central recognition molecule in cellular immunity. Its **extracellular** domains are homologous with and thought to be structurally similar to an antibody Fab fragment. Despite the biological importance of the TCR and the ease of bacterial expression of antibody fragments, there are only few reports of TCR-fragment expression in E. coli. In order to understand the difficulties of expressing correctly **folded** TCR fragments in E. coli, we have characterized the expression behavior of single-chain Fv analogs of three different TCRs (scTCR). All of them can be **folded** into the correct conformation in the periplasm of E. coli, yet the extent of correct **folding** varies greatly. In order to overcome the **folding** problems of some of the scTCRs, we have developed a system with enhanced in vivo **folding** capability based on the simultaneous induction of the heat-shock response and over-expression of the E. coli **disulfide** isomerase DsbA at low temperature. We present a model describing the **folding** of the scTCRs in the periplasm of E. coli and possible points of **folding** assistance. The role of the periplasm as an independent **folding** compartment is emphasized and the existence of a general periplasmic chaperone is postulated. We have also shown that a bivalent scTCR, dimerized in vivo with helix-turn-helix modules, can be expressed in a correctly **folded** form.

Correctly **folded T-cell receptor** fragments in the



09149158 20451136 PMID: 10993731

Heterodimeric CD3epsilon gamma **extracellular** domain fragments:  
production, purification and structural analysis.

Kim K S; Sun Z Y; Wagner G; Reinherz E L

Laboratory of Immunobiology, Dana-Farber Cancer Institute and Department  
of Medicine, Boston, MA, 02115, USA.

Journal of molecular biology (ENGLAND) Sep 29 2000, 302 (4) p899-916

, ISSN 0022-2836 Journal Code: 2985088R

Contract/Grant No.: AI19807; AI; NIAID; AI37581; AI; NIAID

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The CD3 polypeptides (epsilon, gamma, and delta) are non-covalently associated signaling subunits of the **T cell receptor** which form non-disulfide linked epsilon gamma and epsilon delta heterodimers. With the goal of investigating their structure, Escherichia coli expression was utilized to produce CD3 ectodomain fragments including the murine CD3epsilon subunit N-terminal Ig-like **extracellular** domain alone or as a single chain construct with that of CD3gamma. The latter links the CD3gamma segment to the C terminus of the CD3epsilon segment via a 26 amino acid peptide (scCD3epsilon gamma26). Although CD3epsilon could be produced at high yield when directed to inclusion bodies, the refolded monomeric CD3epsilon was not native as judged by monoclonal antibody binding using surface plasmon resonance and was largely unstructured by (15)N-(1)H two-dimensional NMR analysis. In contrast, scCD3epsilon gamma26 could be refolded readily into a native state as shown by CD, NMR and mAb reactivity. The linker length between CD3epsilon and CD3gamma is critical since scCD3epsilon gamma16 containing a 16 residue connector failed to generate a stable heterodimer. Collectively, the results demonstrate that: (i) soluble heterodimeric fragments of CD3 can be produced; (ii) cotranslation of CD3 chains insures proper **folding** even in the absence of the conserved ectodomain stalk region (CxxCxE); and (iii) CD3epsilon has a more stable tertiary protein **fold** than CD3gamma.



```

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HIGHLIGHT set on as ''
? b 340
    05feb04 11:36:36 User231882 Session D1277.2
        $0.00      0.072 DialUnits File410
    $0.00 Estimated cost File410
    $0.02 TELNET
    $0.02 Estimated cost this search
    $0.02 Estimated total session cost    0.228 DialUnits

```

File 340:CLAIMS(R)/US Patent 1950-04/Feb 03  
(c) 2004 IFI/CLAIMS(R)  
\*File 340: Annual reload and classification updates delayed due  
to processing issues.

Set	Items	Description
? s leucine(w)zipper		
	4141	LEUCINE
	4209	ZIPPER
S1	162	LEUCINE (W) ZIPPER
? s fos		
S2	313	FOS
? s s1 and s2		
	162	S1
	313	S2
S3	22	S1 AND S2
? s c(w)jun		
	1031520	C
	5618	JUN
S4	152	C(W) JUN
? s c(w)fos		
	1031520	C
	313	FOS
S5	127	C(W) FOS
? s s3 and s4		
	22	S3
	152	S4
S6	10	S3 AND S4
? s s6 and py<=1998		
	10	S6
	3123744	PY<=1998
S7	4	S6 AND PY<=1998
? t s7/3,k,ab/1-4		

7/3,K,AB/1  
DIALOG(R)File 340:CLAIMS(R)/US Patent  
(c) 2004 IFI/CLAIMS(R). All rts. reserv.

Dialog Acc No: 2856773 IFI Acc No: 9717689  
Document Type: C  
USE OF PAIRS OF **LEUCINE ZIPPER** PEPTIDES IN IMMUNOASSAY METHODS  
Inventors: Auerbach Bernhard (DE); Bosslet Klaus (DE); Hermentin Peter (DE)  
; Muller Rolf (DE); Pfleiderer Peter (DE); Sedlacek Hans Harald (DE)  
Assignee: Behringwerke AG DE  
Assignee Code: 08592 Document Type: REASSIGNED  
Publication (No,Date), Applic (No,Date):  
US 5643731 19970701 US 95467943 19950606  
Publication Kind: A  
Calculated Expiration: 20140701  
(Cited in 002 later patents) Document Type: CERTIFICATE OF CORRECTION  
Certificate of Correction Date: 19980721



Continuation Pub(No),Applic(No,Date): ABANDONED US 91805687  
19911212; ABANDONED US 9327587 19930305; ABANDONED  
US 94182390 19940118; ABANDONED US 94342001  
19941116  
Priority Applic(No,Date): DE 4040669 19901219

Abstract: The invention relates to a method of using a pair of **leucine zipper** peptides for in vitro diagnosis, in particular, for the immunochemical detection and determination of an analyte in a biological liquid. In one method, the first **leucine zipper** peptide is immobilized by attaching it to a solid support, the second **leucine zipper** peptide is coupled to a specific binding partner for the analyte, the two peptides are brought into contact, the sample of the biological liquid is brought into contact with the immobilized first peptide and the specific binding partner for the analyte, and the amount of analyte bound to the binding partner is determined. The **leucine zipper** peptides are preferably v-fos and c-jun.

USE OF PAIRS OF **LEUCINE ZIPPER** PEPTIDES IN IMMUNOASSAY METHODS  
Publication (No,Date), Applic (No,Date):  
...19970701

Abstract: The invention relates to a method of using a pair of **leucine zipper** peptides for in vitro diagnosis, in particular, for the immunochemical detection and determination of an analyte in a biological liquid. In one method, the first **leucine zipper** peptide is immobilized by attaching it to a solid support, the second **leucine zipper** peptide is coupled to a specific binding partner for the analyte, the two peptides are...  
...the analyte, and the amount of analyte bound to the binding partner is determined. The **leucine zipper** peptides are preferably v-fos and c-jun.

Exemplary Claim: ...detection and determination of an analyte in a biological liquid using a pair of complementary **leucine zipper** peptides, wherein the first **leucine zipper** peptide of the pair of **leucine zipper** peptides specifically binds to the second **leucine zipper** peptide of the pair of **leucine zipper** peptides, comprising the steps of: (a) immobilizing said first **leucine zipper** peptide on a solid phase; (b) coupling said second **leucine zipper** peptide to a specific binding partner for the analyte; (c) contacting the immobilized first **leucine zipper** peptide with the second **leucine zipper** peptide coupled to the specific binding partner for the analyte, thereby immobilizing the specific binding...

Non-exemplary Claims: ...3. The method of claim 1, wherein the first **leucine zipper** peptide is one of v-Fos and c-Jun and the second **leucine zipper** peptide is the other  
...

...detection and determination of an analyte in a biological liquid using a pair of complementary **leucine zipper** peptides, wherein the first **leucine zipper** peptide of the pair of **leucine zipper** peptides specifically binds to the second **leucine zipper** peptide of the pair of **leucine zipper** peptides, comprising the steps of: (a) coupling said first **leucine zipper** peptide to a signal-emitting component; (b) coupling said second **leucine zipper** peptide to a first specific binding partner for the analyte; (c) contacting the first **leucine zipper** peptide coupled to the signal-emitting component with the second **leucine zipper** peptide coupled to the specific binding partner for the analyte to form a complex; (d...



- ...6. The method of claim 4, wherein the first **leucine zipper** peptide is one of **v-Fos** and **c-Jun** and the second **leucine zipper** peptide is the other...
- ...in a biological liquid, consisting essentially of: a solid phase; and a pair of complementary **leucine zipper** peptides consisting of a first **leucine zipper** peptide and a second **leucine zipper** peptide, wherein the first **leucine zipper** peptide of the pair of **leucine zipper** peptides specifically binds to the second **leucine zipper** peptide of the pair of **leucine zipper** peptides; wherein the first **leucine zipper** peptide is immobilized on said solid phase, the second **leucine zipper** peptide is coupled to a specific binding partner for the analyte, and the second **leucine zipper** peptide is complexed with the first **leucine zipper** peptide...
- ...determination of an analyte in a biological liquid, consisting essentially of a pair of complementary **leucine zipper** peptides consisting of a first **leucine zipper** peptide and a second **leucine zipper** peptide, wherein the first **leucine zipper** peptide of the pair of **leucine zipper** peptides specifically binds to the second **leucine zipper** peptide of the pair of **leucine zipper** peptides, wherein the first **leucine zipper** peptide is coupled to signal-emitting component and the second **leucine zipper** peptide is coupled to a specific binding partner for the analyte...
- ...being bound to the specific binding partner, wherein the improvement comprises: (a) immobilizing a first **leucine zipper** peptide of a pair of complementary **leucine zipper** peptides to a solid phase, wherein the first **leucine zipper** peptide of the pair of **leucine zipper** peptides specifically binds to a second **leucine zipper** peptide of the pair of **leucine zipper** peptides; (b) coupling said second **leucine zipper** peptide to a specific binding partner for the analyte; and (c) contacting the first **leucine zipper** peptide with the second **leucine zipper** peptide to immobilize said specific binding partner for the analyte to the solid phase...second specific binding partner for the analyte, wherein the improvement comprises: (a) coupling a first **leucine zipper** peptide of a pair of complementary **leucine zipper** peptides to a signal-emitting component, wherein the first **leucine zipper** peptide of the pair of **leucine zipper** peptides specifically binds to a second **leucine zipper** peptide of the pair of **leucine zipper** peptides; (b) coupling said second **leucine zipper** peptide to the second specific binding partner for the analyte; and (c) contacting the first **leucine zipper** peptide with the second **leucine zipper** peptide, thereby attaching the signal-emitting component to the second specific binding partner for the...
- ...the signal in an immunoassay for an analyte, comprising the steps of: (a) coupling first **leucine zipper** peptides of a pair of **leucine zipper** peptides to a signal-emitting component, wherein the first **leucine zipper** peptides of the pair of **leucine zipper** peptides specifically binds to a second **leucine zipper** peptides; (b) coupling more than one molecule of the second **leucine zipper** peptide to a second specific binding partner which specifically binds to a first specific binding partner which specifically binds to the analyte; (c) contacting the first **leucine zipper** peptides coupled to the signal-emitting components with the second **leucine zipper** peptides coupled to the specific binding partner to form a complex; (d) contacting a sample...



...13. The method of claim 11, wherein the first **leucine zipper** peptide is one of **v-Fos** and **c-Jun** and the second **leucine zipper** peptide is the other...  
...second specific binding partner is coupled to the more than one molecules of the second **leucine zipper** peptide via a carrier molecule.

7/3,K,AB/2

DIALOG(R) File 340:CLAIMS(R)/US Patent  
(c) 2004 IFI/CLAIMS(R). All rts. reserv.

Dialog Acc No: 2852080 IFI Acc No: 9716260  
Document Type: C  
FUNCTIONAL ANTAGONISM BETWEEN PROTO-ONCOPROTEIN **C-JUN** AND HORMONE RECEPTORS; METHOD FOR SELECTING A COMPOUND WHICH DISRUPTS FUNCTION OF ACTIVATOR PROTEIN 1 BUT DOES NOT PROMOTE TRANSCRIPTIONAL ACTIVATION OF STEROID HORMONE RESPONSIVE GENE; FOR SELECTING COMPOUNDS FOR TREATING CELLS UNDERGOING UNCONTROLLED GROWTH  
Inventors: Evans Ronald M (US); Schule Ronald (DE)  
Assignee: Salk Institute for Biological Studies  
Assignee Code: 73756  
Publication (No,Date), Applic (No,Date):  
US 5639592 19970617 US 9430330 19940503  
Publication Kind: A  
Calculated Expiration: 20140617  
(Cited in 004 later patents)  
Cont.-in-part Pub(No),Applic(No,Date): ABANDONED US  
90586187 19900921  
PCT Pub(No,Date),Applic(No,Date): WO 925447 19920402 WO  
91US6848 19910920  
Section 371: 19940503  
Section 102(e):19940503  
Priority Applic(No,Date): US 9430330 19940503; US 90586187 19900921

Abstract: Hormone receptors and the transcription factor Jun/AP-1 have been shown to reciprocally repress one another by a mechanism which is independent of DNA binding. For example, over-expression of AP-1 represses glucocorticoid-induced activation of genes carrying a functional glucocorticoid response element. Conversely, glucocorticoid has been shown to repress the transcriptional activation of genes which are controlled by promoters which contain the AP-1 binding site. In addition, methods are disclosed for selecting compounds useful for treating cells undergoing uncontrolled proliferation, such compounds being capable of disrupting the function of AP-1, but display substantially no ability to promote the transcriptional activation of hormone responsive genes.

FUNCTIONAL ANTAGONISM BETWEEN PROTO-ONCOPROTEIN **C-JUN** AND HORMONE RECEPTORS...

Publication (No,Date), Applic (No,Date):

...19970617

...PCT Pub(No,Date),Applic(No,Date): 19920402

Non-exemplary Claims: ...steroid hormone-responsive gene, said method comprising: administering to said system a peptide comprising the **leucine zipper** region of **c-Jun** in an amount ...

11. The method according to claim 10 wherein the molar ratio of the **leucine zipper** region of **c-Jun** to steroid hormone receptor falls in the range of about 0.5:1 up to...

...according to claim 12 wherein said AP-1-responsive gene is a collagenase gene, a **c-Jun** gene, a **c-Fos** gene, an immune-response gene, or a retinoic acid receptor-alpha gene...



7/3,K,AB/3

DIALOG(R) File 340:CLAIMS(R)/US Patent

(c) 2004 IFI/CLAIMS(R). All rts. reserv.

Dialog Acc No: 2791522 IFI Acc No: 9631248

Document Type: C

BIFUNCTIONAL ANTIBODIES AND METHOD OF PREPARING SAME; FIRST FAB FRAGMENT AND SECOND FAB FRAGMENT STABLY ASSOCIATED THROUGH COMPLEMENTARY DOMAINS WHICH BIND TO FORM **LEUCINE ZIPPER**, FORMING ANTIBODY CONSTRUCT CAPABLE OF BINDING TO TWO ANTIGENIC SITES

Inventors: Curtis Peter J (US)

Assignee: Wistar Institute of Anatomy and Biology The

Assignee Code: 92890

Publication (No,Date), Applic (No,Date):

US ~~5582996~~ **19961210** US 94250656 19940527 ✓

Publication Kind: A

Calculated Expiration: 20131210

(Cited in 002 later patents) Document Type: CERTIFICATE OF CORRECTION

Certificate of Correction Date: 19970610

Continuation Pub(No),Applic(No,Date): ABANDONED

US 92917074

19920731

Cont.-in-part Pub(No),Applic(No,Date): ABANDONED

US

90622983 19901204

Priority Applic(No,Date): US 94250656 19940527; US 92917074 19920731;

US 90622983 19901204

Abstract: A recombinant antibody capable of binding to two different antigenic sites, contains Fab fragments from the same or, preferably, different antibodies, which are brought into association by complementary interactive domains which have been inserted into a region of the antibody heavy chain constant region.

...FAB FRAGMENT AND SECOND FAB FRAGMENT STABLY ASSOCIATED THROUGH COMPLEMENTARY DOMAINS WHICH BIND TO FORM **LEUCINE ZIPPER**, FORMING ANTIBODY CONSTRUCT CAPABLE OF BINDING TO TWO ANTIGENIC SITES  
Publication (No,Date), Applic (No,Date):

...**19961210**

Exemplary Claim: ...but not to itself, wherein the first and second complementary domains bind to form a **leucine zipper** and to stably associate said first Fab fragment and said second Fab fragment into a...

Non-exemplary Claims: ...but not to itself, whereby the first and second complementary domains interact to form a **leucine zipper** to associate the first and second modified Fab regions into a single antibody construct capable...

...the first complementary domain, wherein the first and second complementary domains bind to form a **leucine zipper** and to stably associate said first Fab fragment and said second Fab fragment into a...

...antibody construct capable of binding to two antigenic sites, wherein said first domain is the **leucine zipper** region of c-**fos** oncogene and said second domain is the **leucine zipper** region of c-**jun** oncogene... ✓

...but not to itself, whereby the first and second complementary domains interact to form a **leucine zipper** to associate the first and second modified Fab regions into a single antibody construct capable of binding to two antigenic sites, wherein the complementary domains are



selected from the **leucine zipper** regions of the oncogenes **c-fos** and **c-jun**.

7/3,K,AB/4

DIALOG(R) File 340:CLAIMS(R)/US Patent

(c) 2004 IFI/CLAIMS(R). All rts. reserv.

Dialog Acc No: 2666744 IFI Acc No: 9529139

Document Type: C

TRANSDOMINANT NEGATIVE PROTO-ONCOGENE; GENETIC ENGINEERING

Inventors: Verma Inder M (US); Wisdom Ronald M (US); Yen Jong-Young J (US)

Assignee: Salk Institute for Biological Studies

Assignee Code: 73756

Publication (No,Date), Applic (No,Date):

US 5470736 19951128 US 94218686 19940328

Publication Kind: A

Calculated Expiration: 20121128

(Cited in 005 later patents)

Continuation Pub(No),Applic(No,Date): ABANDONED

US 91710862

19910610

Priority Applic(No,Date): US 94218686 19940328; US 91710862 19910610

Abstract: The present invention provides polynucleotide and polypeptide sequences for a trans-repressing protein of the **Fos** protooncogene family, where the polypeptide is characterized by having a **leucine zipper** domain and forming a heterodimer with a Jun related protein. This heterodimer is capable of binding to an AP-1 site and suppressing transcriptional transactivation of a promoter containing the AP-1 site.

Publication (No,Date), Applic (No,Date):

...19951128

Abstract: The present invention provides polynucleotide and polypeptide sequences for a trans-repressing protein of the **Fos** protooncogene family, where the polypeptide is characterized by having a **leucine zipper** domain and forming a heterodimer with a Jun related protein. This heterodimer is capable of...

Exemplary Claim: ...encoding a trans-repressing FosB2 protein, wherein the protein is characterized by: (a) having a **leucine zipper** domain; and (b) forming a heterodimer with a Jun related protein, wherein the heterodimer is...

Non-exemplary Claims: ...of claim 1, wherein the Jun related protein is selected from the group consisting of **c-Jun**, Jun B and Jun D

...

?



SYSTEM:OS - DIALOG OneSearch

File 155:MEDLINE(R) 1966-2004/Feb W1

(c) format only 2004 The Dialog Corp.

\*File 155: Medline is updating again (12-22-2003).

Please see HELP NEWS 154, for details.

File 55:Biosis Previews(R) 1993-2004/Jan W4

(c) 2004 BIOSIS

File 34:SciSearch(R) Cited Ref Sci 1990-2004/Jan W4

(c) 2004 Inst for Sci Info

\*File 34: New prices as of 1/1/2004 per Information Provider request. See HELP RATES 34.

File 434:SciSearch(R) Cited Ref Sci 1974-1989/Dec

(c) 1998 Inst for Sci Info

\*File 434: New prices as of 1/1/2004 per Information Provider request. See HELP RATES434.

File 340:CLAIMS(R)/US Patent 1950-04/Feb 03

(c) 2004 IFI/CLAIMS(R)

\*File 340: Annual reload and classification updates delayed due to processing issues.

Set	Items	Description	
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? s e?(w)coli

>>>File 155 processing for E? stopped at EBDAS

>>>File 55 processing for E? stopped at EBCOA

>>>File 34 processing for E? stopped at EASTLAND

<-----User Break----->

u!

? s escherichia(w)coli

582844 ESCHERICHIA

625054 COLI

S1 579144 ESCHERICHIA(W)COLI

? s express?

Processing

S2 2930609 EXPRESS?

? s s1 and s2

579144 S1

2930609 S2

S3 191284 S1 AND S2

? s advantage

S4 159658 ADVANTAGE

? s s3 and s4

191284 S3

159658 S4

S5 999 S3 AND S4

? s high(5n)yield??

4380765 HIGH

778319 YIELD??

S6 63965 HIGH(5N)YIELD??

? s s5 and s6

999 S5

63965 S6

S7 28 S5 AND S6

? rd

>>>Duplicate detection is not supported for File 340.

>>>Records from unsupported files will be retained in the RD set.

...completed examining records

S8 14 RD (unique items)

? s s8 and py<=1998

Processing

14 S8

33644571 PY<=1998

S9 7 S8 AND PY<=1998



? t s9/3,k,ab/1-7

9/3,K,AB/1 (Item 1 from file: 155)

DIALOG(R) File 155:MEDLINE(R)

(c) format only 2004 The Dialog Corp. All rts. reserv.

10373856 96178941 PMID: 8606152

The regulatory characteristics of yeast fructose-1,6-bisphosphatase confer only a small selective **advantage**.

Navas M A; Gancedo J M

Instituto de Investigaciones Biomedicas, Consejo Superior de Investigaciones Cientificas, Madrid, Spain.

Journal of bacteriology (UNITED STATES) Apr 1996, 178 (7)

p1809-12, ISSN 0021-9193 Journal Code: 2985120R

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The question of how the loss of regulatory mechanisms for a metabolic enzyme would affect the fitness of the corresponding organism has been addressed. For this, the fructose-1,6-bisphosphatase (FbPase) from *Saccharomyces cerevisiae* has been taken as a model. Yeast strains in which different controls on FbPase (catabolite repression and inactivation; inhibition by fructose-2,6-bisphosphate and AMP) have been removed have been constructed. These strains **express** during growth on glucose either the native yeast FbPase, the *Escherichia coli* FbPase which is insensitive to inhibition by fructose-2,6-bisphosphate, or a mutated *E. coli* FbPase with low sensitivity to AMP. **Expression** of the heterologous FbPases increases the fermentation rate of the yeast and its generation time, while it decreases its growth **yield**. In the strain containing **high** levels of an unregulated bacterial FbPase, cycling between fructose-6-phosphate and fructose-1,6-bisphosphate reaches 14%. It is shown that the regulatory mechanisms of FbPase provide a slight but definite competitive **advantage** during growth in mixed cultures.

The regulatory characteristics of yeast fructose-1,6-bisphosphatase confer only a small selective **advantage**.

Apr 1996,

... by fructose-2,6-bisphosphate and AMP) have been removed have been constructed. These strains **express** during growth on glucose either the native yeast FbPase, the *Escherichia coli* FbPase which is insensitive to inhibition by fructose-2,6-bisphosphate, or a mutated *E. coli* FbPase with low sensitivity to AMP. **Expression** of the heterologous FbPases increases the fermentation rate of the yeast and its generation time, while it decreases its growth **yield**. In the strain containing **high** levels of an unregulated bacterial FbPase, cycling between fructose-6-phosphate and fructose-1,6...

... It is shown that the regulatory mechanisms of FbPase provide a slight but definite competitive **advantage** during growth in mixed cultures.

; Adenosine Monophosphate--metabolism--ME; *Escherichia coli*  
--enzymology--EN; Fructose-Bisphosphatase--genetics--GE; Gene **Expression**  
Regulation, Fungal; Glucose--metabolism--ME; Recombinant Proteins  
--genetics--GE; Recombinant Proteins--metabolism--ME; *Saccharomyces cerevisiae*...

9/3,K,AB/2 (Item 2 from file: 155)

DIALOG(R) File 155:MEDLINE(R)

(c) format only 2004 The Dialog Corp. All rts. reserv.

10344444 96146908 PMID: 8588916

Single-step purification of a thermostable DNA polymerase **expressed** in *Escherichia coli*.



Desai U J; Pfaffle P K  
Department of Life Sciences, Indiana State University, Terre Haute 47809,  
USA.

BioTechniques (UNITED STATES) Nov 1995, 19 (5) p780-2, 784,  
ISSN 0736-6205 Journal Code: 8306785

Document type: Technical Report

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The coding region of the gene for Taq DNA polymerase has been cloned into the common vector pUC18. Using a single-step procedure, large amounts of active enzyme can be purified from *Escherichia coli* carrying this construct. This procedure takes **advantage** of the thermostable properties of the DNA polymerase. This simple procedure gives very **high yields** of essentially homogeneous, highly active enzyme suitable for use in molecular biological applications. Yields are over two orders of magnitude greater than available with current methods.

Single-step purification of a thermostable DNA polymerase **expressed** in *Escherichia coli*.

Nov 1995,

... pUC18. Using a single-step procedure, large amounts of active enzyme can be purified from *Escherichia coli* carrying this construct. This procedure takes **advantage** of the thermostable properties of the DNA polymerase. This simple procedure gives very **high yields** of essentially homogeneous, highly active enzyme suitable for use in molecular biological applications. Yields are...

Descriptors: DNA-Directed DNA Polymerase--isolation and purification--IP; \**Escherichia coli*--genetics--GE; Base Sequence; Cloning, Molecular; DNA-Directed DNA Polymerase--genetics--GE; Electrophoresis, Polyacrylamide Gel; Enzyme Stability; *Escherichia coli* --enzymology--EN; Gene **Expression**; Genetic Vectors; Heat; Molecular Sequence Data; Polymerase Chain Reaction; Taq Polymerase

9/3,K,AB/3 (Item 3 from file: 155)  
DIALOG(R) File 155:MEDLINE(R)  
(c) format only 2004 The Dialog Corp. All rts. reserv.

08191784 94257706 PMID: 8199219

Purification of recombinant adenomatous polyposis coli polypeptide chains from *E. coli* extracts by continuous-elution electrophoresis.

Kraus C; Klein E; Ballhausen W G

Institut fuer Humangenetik, Universitaet Erlangen-Nuernberg, Germany.

Applied and theoretical electrophoresis - the official journal of the International Electrophoresis Society (ENGLAND) 1993, 3 (6)  
p271-5, ISSN 0954-6642 Journal Code: 8915308

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

A polypeptide chain encoded by the exons 1-3 of the human adenomatous polyposis coli (APC) tumor suppressor gene was **expressed** as a maltose binding fusion protein (MBP) in *E. coli* to be used for immunization purposes. It turned out, that the APC-MBP fusion product of 60 kDa was deposited in bacterial inclusion bodies and, in addition, it was not retarded by an amylose affinity column, most probably due to an altered conformation of the chimeric molecule. For this reason, we established an alternative purification scheme, which took **advantage** of SDS-extraction followed by a high-resolution two-step continuous-elution electrophoresis (CEE) procedure. This purification method allowed us to obtain **high yields** of pure human APC exon 1-3-encoded proteins. The final yield of the pure APC polypeptide chains was estimated to represent 5-8% of the amount of SDS-extracted *E. coli* lysate subjected to the first cycle of CEE. The purified APC molecules were successfully used



for the development of specific antibodies. The CEE procedure described here represents a general purification method which is valuable in cases where fusion proteins are deposited as inclusion bodies in bacteria, or if affinity chromatography is precluded due to a conformation-induced lack of ligand binding of the chimeric molecule.

1993,

... the exons 1-3 of the human adenomatous polyposis coli (APC) tumor suppressor gene was **expressed** as a maltose binding fusion protein (MBP) in E. coli to be used for immunization...

...of the chimeric molecule. For this reason, we established an alternative purification scheme, which took **advantage** of SDS-extraction followed by a high-resolution two-step continuous-elution electrophoresis (CEE) procedure. This purification method allowed us to obtain **high yields** of pure human APC exon 1-3-encoded proteins. The final yield of the pure...

; Base Sequence; Cloning, Molecular; Electrophoresis--methods--MT; **Escherichia coli**; Molecular Sequence Data; Neoplasm Proteins --biosynthesis--BI; Neoplasm Proteins--genetics--GE; Peptide Synthesis; Peptides--genetics...

9/3,K,AB/4 (Item 4 from file: 155)  
DIALOG(R)File 155:MEDLINE(R)  
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07281382 92144101 PMID: 1368756

Utilization of trans-activated T4 uvsY regulatory signal for a **high yield** of cloned gene products.

Noguchi T; Takahashi H

Research Laboratories, Yamasa Shoyu Co., Ltd., Chiba, Japan.

Agricultural and biological chemistry (JAPAN) Oct 1991, 55 (10)

p2497-506, ISSN 0002-1369 Journal Code: 0370452

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

We established an efficient system for a high-level production of foreign gene products in **Escherichia coli** using a trans-activated gene **expression** under the control of a phage T4 regulatory signal cloned in a plasmid by T4 phage infection. The transcriptional and translational signal of the T4 uvsY gene cloned in a plasmid was fused translationally with the coding region of the lacZ gene. When E. coli cells carrying the uvsY-lacZ plasmid were infected with cytosine-substituting T4 phage at a multiplicity of infection of 5, the amount of beta-galactosidase increased about 2-fold (trans-activation) over that without phage infection. We examined conditions for the high-level production of a trans-activated gene product. We found that a large number of T4-infected cells in a lysis-inhibition state could be obtained by a low multiplicity of infection with cytosine-substituting T4 phage. Thus it is now possible to attain a **high yield** of the trans-activated gene products. We discuss the **advantage** of the trans-activated T4 uvsY regulatory signal for production of foreign products.

Utilization of trans-activated T4 uvsY regulatory signal for a **high yield** of cloned gene products.

Oct 1991,

We established an efficient system for a high-level production of foreign gene products in **Escherichia coli** using a trans-activated gene **expression** under the control of a phage T4 regulatory signal cloned in a plasmid by T4...

... of infection with cytosine-substituting T4 phage. Thus it is now



possible to attain a **high yield** of the trans-activated gene products. We discuss the **advantage** of the trans-activated T4 uvsY regulatory signal for production of foreign products.

Descriptors: Bacterial Proteins--genetics--GE; \*Gene **Expression** Regulation, Bacterial--genetics--GE; \*Regulatory Sequences, Nucleic Acid --genetics--GE; \*T-Phages--genetics--GE; \*Trans...  
; Amino Acid Sequence; Base Sequence; Cloning, Molecular; Cytosine --physiology--PH; DNA, Recombinant--genetics--GE; **Escherichia coli**--genetics--GE; Molecular Sequence Data; Promoter Regions (Genetics)--genetics--GE

9/3,K,AB/5 (Item 5 from file: 155)  
DIALOG(R) File 155:MEDLINE(R)  
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07026078 91266933 PMID: 2050135

Single-step purification and structural characterization of human interleukin-6 produced in **Escherichia coli** from a T7 RNA polymerase **expression** vector.

Arcone R; Pucci P; Zappacosta F; Fontaine V; Malorni A; Marino G; Ciliberto G

Dipartimento di Biochimica e Biotecnologie Mediche, II Facolta di Medicina e Chirurgia, Universita di Napoli, Italy.

European journal of biochemistry / FEBS (GERMANY) Jun 15 1991,  
198 (3) p541-7, ISSN 0014-2956 Journal Code: 0107600

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Human interleukin-6 or B-cell stimulatory factor-2 is a cytokine involved in acute phase and immune response. Cloning of cDNA for human interleukin-6 in the pT7.7 **expression** plasmid under the control of a bacteriophage T7 RNA polymerase promoter system allows rapid production of the cytokine in **Escherichia coli**. Upon cell induction with isopropyl thiogalactopyranoside, recombinant human interleukin-6 is overexpressed and forms insoluble inclusion bodies. Solubilization of the protein with 6 M guanidine hydrochloride and refolding in the presence of a reduction/oxidation system results in a quantitative recovery of recombinant human interleukin-6. This material is already 70% pure and can be further purified to homogeneity with a single passage over a weak anionic-exchange column. Extended structural characterization of the purified protein by electrospray mass spectrometry, automated Edman degradation and peptide mapping by high-pressure liquid chromatography/fast-atom-bombardment mass spectrometry demonstrates that recombinant human interleukin-6 is identical to the natural protein both in amino acid sequence and S-S bridge content. However, it contains a minor component accounting for about 20% of the entire translated protein which exhibits a Met-Ala dipeptide extension at the N-terminus. Purified recombinant human interleukin-6 is biologically active because it is able to induce at least 70-fold the human C-reactive promoter transfected in human hepatoma Hep 3B cells and is stable for several months in 10% glycerol at 4 degrees C. The **expression** system described in the present work has the main **advantage** of producing a **high yield** of recombinant human interleukin-6 (about 25 mg/l) combined with a very simple purification scheme.

Single-step purification and structural characterization of human interleukin-6 produced in **Escherichia coli** from a T7 RNA polymerase **expression** vector.

Jun 15 1991,

... phase and immune response. Cloning of cDNA for human interleukin-6 in the pT7.7 **expression** plasmid under the control of a bacteriophage T7 RNA polymerase promoter system allows rapid production of the cytokine in

2/4/04



**Escherichia coli** . Upon cell induction with isopropyl thiogalactopyranoside, recombinant human interleukin-6 is overexpressed and forms insoluble...

... cells and is stable for several months in 10% glycerol at 4 degrees C. The **expression** system described in the present work has the main **advantage** of producing a **high yield** of recombinant human interleukin-6 (about 25 mg/l) combined with a very simple purification...

Descriptors: DNA-Directed RNA Polymerases--genetics--GE; \***Escherichia coli**--genetics--GE; \*Interleukin-6--isolation and purification--IP; \*T-Phages--genetics--GE

9/3,K,AB/6 (Item 6 from file: 155)  
DIALOG(R) File 155:MEDLINE(R)  
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06853210 91093287 PMID: 1985969

Cloning, sequencing, and overexpression of genes for ribosomal proteins from *Bacillus stearothermophilus*.

Ramakrishnan V; Gerchman S E  
Biology Department, Brookhaven National Laboratory, Upton, New York 11973.

Journal of biological chemistry (UNITED STATES) Jan 15 1991, 266

(2) p880-5, ISSN 0021-9258 Journal Code: 2985121R

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Although a low resolution model for the arrangement of the proteins of the small and large ribosomal subunits is known, a detailed mechanistic understanding of the function of the ribosome awaits a high resolution structure of its components. While crystals have been obtained of several ribosomal proteins from *Bacillus stearothermophilus*, determination of atomic resolution structures of these proteins is impeded by the difficulty of obtaining large amounts of native proteins for crystallographic or NMR studies. We describe here the cloning and overexpression in **Escherichia coli** of the genes for ribosomal proteins S5, L6, L9, and L18 from *B. stearothermophilus*. S5 is extremely toxic to *E. coli* when overexpressed, and we have taken **advantage** of a new tightly regulated **expression** system to obtain **high yields** (more than 100 mg of pure protein/liter of culture) of this protein. The *B. stearothermophilus* S5 produced in *E. coli* crystallizes, and the crystals are identical to those obtained from the native protein. The crystals diffract to 2-A resolution.

Jan 15 1991,

... native proteins for crystallographic or NMR studies. We describe here the cloning and overexpression in **Escherichia coli** of the genes for ribosomal proteins S5, L6, L9, and L18 from *B. stearothermophilus*. S5 is extremely toxic to *E. coli* when overexpressed, and we have taken **advantage** of a new tightly regulated **expression** system to obtain **high yields** (more than 100 mg of pure protein/liter of culture) of this protein. The B...

Descriptors: *Bacillus stearothermophilus*--metabolism--ME; \*Gene **Expression** Regulation, Bacterial; \*Genes, Bacterial; \*Ribosomal Proteins--genetics--GE...; *Bacillus stearothermophilus*--genetics--GE; Base Sequence; Cloning, Molecular; DNA, Bacterial--genetics--GE; Electrophoresis, Polyacrylamide Gel; **Escherichia coli**--genetics--GE; Molecular Sequence Data; Polymerase Chain Reaction

9/3,K,AB/7 (Item 1 from file: 34)  
DIALOG(R) File 34:SciSearch(R) Cited Ref Sci



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04505586 Genuine Article#: TH829 Number of References: 27

Title: SECRETION OF BIOLOGICALLY-ACTIVE HUMAN PROAPOLIPOPROTEIN A-I IN A  
BACULOVIRUS-INSECT CELL SYSTEM - PROTECTION FROM DEGRADATION BY  
PROTEASE INHIBITORS (Abstract Available)

Author(s): PYLE LE; BARTON P; FUJIWARA Y; MITCHELL A; FIDGE N

Corporate Source: BAKER MED RES INST, LIPOPROT & ATHEROSCLEROSIS  
UNIT/PRAHRAN/VIC 3181/AUSTRALIA/; BAKER MED RES INST, LIPOPROT &  
ATHEROSCLEROSIS UNIT/PRAHRAN/VIC 3181/AUSTRALIA/; SWINBURNE UNIV  
TECHNOL/HAWTHORN/VIC 3122/AUSTRALIA/

Journal: JOURNAL OF LIPID RESEARCH, 1995, V36, N11 (NOV), P2355-2361

ISSN: 0022-2275

Language: ENGLISH Document Type: ARTICLE

Abstract: Studies of the structure and function of apolipoprotein A-I  
(apoA-I) often require its purification by delipidation of high density  
lipoprotein isolated from large quantities of human plasma and  
separation of apoA-I from other plasma apolipoproteins. To reduce the  
need for extensive purification procedures, we have developed an insect  
cell/baculovirus **expression** system for the production and  
secretion of human proapoA-I. The recombinant baculovirus containing  
full-length human apoA-I cDNA, when introduced into Spodoptera  
frugiperda, directs the synthesis of preproapoA-I, which is  
subsequently secreted into the growth medium as proapoA-I, indicating  
correct processing of the signal peptide during secretion. To prevent  
the extensive degradation of secreted proapoA-I, leupeptin and  
pepstatin A were added to the serum free cell culture medium. The  
protein was simply purified by filtration of the medium, which  
contained up to 80 mg/l proapoA-I, followed by chromatography on  
phenyl-sepharose CL-4B. The resultant proapoA-I was found to bind lipid  
and to activate lecithin:cholesterol acyltransferase as effectively as  
apoA-I from human plasma. The **advantage** of this **expression**  
system is the ease of purification of intact, biologically active  
apoA-I in **high yield**.

, 1995

...Abstract: To reduce the need for extensive purification procedures, we  
have developed an insect cell/baculovirus **expression** system for  
the production and secretion of human proapoA-I. The recombinant  
baculovirus containing full...

...and to activate lecithin:cholesterol acyltransferase as effectively as  
apoA-I from human plasma. The **advantage** of this **expression**  
system is the ease of purification of intact, biologically active  
apoA-I in **high yield**.

...Identifiers--LECITHIN-CHOLESTEROL ACYLTRANSFERASE; VIRUS  
**EXPRESSION** VECTORS; HIGH-DENSITY-LIPOPROTEIN; HUMAN  
APOLIPOPROTEIN-E; **ESCHERICHIA-COLI**; BINDING DOMAIN;  
CONVERSION; ANTIBODIES; PARTICLES; RECEPTOR

...Research Fronts: LIPOPROTEIN RECEPTOR; LDL METABOLISM; HEPG2 CELLS;  
CHOLESTEROL-FED RABBITS; TRANSGENIC MICE)

93-2767 001 (BACULOVIRUS **EXPRESSION** SYSTEM; INSECT CELLS;  
AUTOGRAPHICA-CALIFORNICA NUCLEAR POLYHEDROSIS-VIRUS; RECOMBINANT VIRAL  
INSECTICIDES)

93-3796 001 (HIGH...

?



SYSTEM:OS - DIALOG OneSearch

File 155:MEDLINE(R) 1966-2004/Jan W3

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\*File 155: Medline is updating again (12-22-2003).

Please see HELP NEWS 154, for details.

File 55:Biosis Previews(R) 1993-2004/Jan W2

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File 34:SciSearch(R) Cited Ref Sci 1990-2004/Jan W2

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\*File 34: New prices as of 1/1/2004 per Information Provider request. See HELP RATES 34.

File 434:SciSearch(R) Cited Ref Sci 1974-1989/Dec

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\*File 434: New prices as of 1/1/2004 per Information Provider request. See HELP RATES434.

File 340:CLAIMS(R)/US Patent 1950-03/Jan 20

(c) 2004 IFI/CLAIMS(R)

\*File 340: Enter HELP NEWS340 & HELP ALERTS340 for search, display & Alert information.

Set Items Description

? s TCR or T(2n)receptor

45365 TCR

4710039 T

1632467 RECEPTOR

94552 T(2N)RECEPTOR

S1 112057 TCR OR T(2N)RECEPTOR

? s disulfide

S2 75854 DISULFIDE

? s s1 and s2

112057 S1

75854 S2

S3 1810 S1 AND S2

? s detrimental

S4 37324 DETRIMENTAL

? s s3 and s4

1810 S3

37324 S4

S5 0 S3 AND S4

? s fold?

S6 714834 FOLD?

? s s3 and s6

1810 S3

714834 S6

S7 129 S3 AND S6

? s unstabl? or aggregat?

119811 UNSTABL?

315431 AGGREGAT?

S8 431131 UNSTABL? OR AGGREGAT?

? s s7 and s8

129 S7

431131 S8

S9 18 S7 AND S8

? rd

>>>Duplicate detection is not supported for File 340.

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...completed examining records

S10 15 RD (unique items)

? t s10/3,k,ab/1-15

10/3,K,AB/1 (Item 1 from file: 155)



11705318 99141420 PMID: 9917415

**Folding**, heterodimeric association and specific peptide recognition of a murine alphabeta T-cell **receptor** expressed in Escherichia coli.

Pecorari F; Tissot A C; Pluckthun A  
Biochemisches Institut, Universitat Zurich, Winterthurerstr. 190, Zurich, CH-8057, Switzerland.

Journal of molecular biology (ENGLAND) Jan 29 1999, 285 (4) p1831-43

, ISSN 0022-2836 Journal Code: 2985088R

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

In a systematic study of the murine T-cell **receptor** UZ3-4, expressed and refolded from inclusion bodies in Escherichia coli, it was found that functional molecules can be obtained only under a very narrow set of conditions. The refolded T-cell **receptor** UZ3-4 specifically recognizes its cognate peptide (from mycobacterial Hsp60) in the context of H-2Db, but not another peptide bound to H-2Db, and the dissociation constant was determined by BIAcore as  $10^{-4}$  M. Using T-cell **receptor** constructs comprising all extracellular domains (ValphaCalpha and VbetaCbeta), found to be necessary for stability of the final product, significant amounts of native molecules were obtained only if the intermolecular Calpha-Cbeta **disulfide** bridge bond was deleted, even though the interaction between the complete alpha and beta-chain was determined to be very weak and fully reversible (KD approximately  $10^{-7}$  to  $10^{-6}$  M). Fusion of Jun and Fos to the constant domains also decreased the folding yield, because of premature association of intermediates leading to aggregation. Furthermore, only in a very narrow set of concentrations of oxidized and reduced glutathione, native **disulfide** bonds dominated. This shows that T-cell **receptor** domains are very prone to **aggregation** and misassociation during **folding**, compounded by incorrect **disulfide** bond formation. Once **folded**, however, the heterodimeric molecule is very stable and could be concentrated to millimolar concentration. Copyright 1999 Academic Press.

**Folding**, heterodimeric association and specific peptide recognition of a murine alphabeta T-cell **receptor** expressed in Escherichia coli.

In a systematic study of the murine T-cell **receptor** UZ3-4, expressed and refolded from inclusion bodies in Escherichia coli, it was found that functional molecules can be obtained only under a very narrow set of conditions. The refolded T-cell **receptor** UZ3-4 specifically recognizes its cognate peptide (from mycobacterial Hsp60) in the context of H...

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...  $10^{-6}$  M). Fusion of Jun and Fos to the constant domains also decreased the **folding** yield, because of premature association of intermediates leading to **aggregation**. Furthermore, only in a very narrow set of concentrations of oxidized and reduced glutathione, native **disulfide** bonds dominated. This shows that T-cell **receptor** domains are



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...; CH; Ligands; Mice; Molecular Sequence Data; Peptides--chemistry--CH; Peptides--metabolism--ME; Protein Conformation; Protein **Folding**; Receptors, Antigen, T-Cell, alpha-beta--genetics--GE; Recombinant Proteins--chemistry--CH; Recombinant Proteins--genetics...

10/3,K,AB/2 (Item 2 from file: 155)  
DIALOG(R) File 155:MEDLINE(R)  
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11106161 97400339 PMID: 9257833

Recombinant soluble alphabeta T cell receptors protect T cells from immune suppression: requirement for **aggregated** multimeric, **disulfide**-linked alphabeta heterodimers.

Paliwal V; Ptak W; Sperl J; Braswell E; Askenase P W  
Department of Internal Medicine, Yale University School of Medicine, New Haven, CT 06520, USA.

Journal of immunology (Baltimore, Md. - 1950) (UNITED STATES) Aug 15 1997, 159 (4) p1718-27, ISSN 0022-1767 Journal Code: 2985117R

Contract/Grant No.: AI-12211; AI; NIAID; AI-26689; AI; NIAID

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Recombinant soluble T cell receptors (sTCR) protected contact sensitivity (CS) effector T cells from down-regulation or immunosuppression. CS-protecting sTCR were released enzymatically from the surface of thymoma cells transfected with cDNAs encoding **TCR** -alpha and -beta extracellular domains that were expressed with a phosphatidylinositol linkage. sTCR affinity purified on anti-**TCR**-alpha and anti-**TCR** -beta mAb columns had identical CS-protective activity, as did sTCR from a CD4+ Th2 clone or from a CD8+ cytotoxic clone. Reduced sTCR alpha- and beta-chains had no CS-protective activity, but this was restored when the **TCR** chains were rejoined into **disulfide** -linked alphabeta heterodimers. sTCR CS protection was Ag nonspecific, MHC unrestricted, and not influenced by the relevant synthetic peptide specific for the **TCR** complexed with appropriate MHC. CS protection may have resided in the sTCR constant region. When heated at 62 degrees C for 30 min, sTCR formed a CS-protecting **aggregate**, with a molecular mass of 481 +/- 37 kDa, corresponding to an alphabeta **TCR** pentamer. HPLC gel filtration essentially confirmed the molecular mass at 516 kDa for the multimer, while the monomer, which was an alphabeta **TCR** heterodimer, had an expected molecular mass of approximately 104 kDa and no bioactivity. In summary, the pentameric sTCR may bind to and activate lymphoid cells, perhaps via constant domains, resulting in protection of CS effector T cells from down-regulation. The ability of sTCR to protect CS effector T cells from down-regulation/suppression, if generalized, could overcome immunosuppression accompanying infectious diseases, particularly AIDS, or in tumors.

Recombinant soluble alphabeta T cell receptors protect T cells from immune suppression: requirement for **aggregated** multimeric, **disulfide**-linked alphabeta heterodimers.

... protecting sTCR were released enzymatically from the surface of thymoma cells transfected with cDNAs encoding **TCR**-alpha and -beta extracellular domains that were expressed with a phosphatidylinositol linkage. sTCR affinity purified on anti-**TCR**-alpha and anti-**TCR** -beta mAb columns had identical CS-protective activity, as did sTCR from a CD4+ Th2...



... alpha- and beta-chains had no CS-protective activity, but this was restored when the TCR chains were rejoined into **disulfide**-linked alphabeta heterodimers. sTCR CS protection was Ag nonspecific, MHC unrestricted, and not influenced by the relevant synthetic peptide specific for the TCR complexed with appropriate MHC. CS protection may have resided in the sTCR constant region. When heated at 62 degrees C for 30 min, sTCR formed a CS-protecting **aggregate**, with a molecular mass of 481 +/- 37 kDa, corresponding to an alphabeta TCR pentamer. HPLC gel filtration essentially confirmed the molecular mass at 516 kDa for the multimer, while the monomer, which was an alphabeta TCR heterodimer, had an expected molecular mass of approximately 104 kDa and no bioactivity. In summary...

...; prevention and control--PC; Disulfides--chemistry--CH; Electrophoresis, Polyacrylamide Gel; Mice; Mice, Inbred CBA; Protein **Folding**; Recombinant Proteins--pharmacology--PD

10/3,K,AB/3 (Item 3 from file: 155)  
DIALOG(R) File 155:MEDLINE(R)  
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06183234 89198838 PMID: 2522980

Production and characterization of monoclonal antibodies to Fc gamma 2a-binding protein isolated from the detergent lysate of a murine macrophagelike cell line, P388D1.

Kagami M; Funatsu Y; Suzuki T

Department of Microbiology, Molecular Genetics, and Immunology, University of Kansas Medical Center, Kansas City 66103.

Journal of leukocyte biology (UNITED STATES) Apr 1989, 45 (4)  
p311-21, ISSN 0741-5400 Journal Code: 8405628

Contract/Grant No.: AI22742; AI; NIAID; CA35977; CA; NCI

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Hybridoma cell lines were produced by fusion of SP2/0 murine myeloma cell line with the spleen cells of Wister rats which were immunized with IgG2a-binding protein isolated from the detergent lysate of a murine macrophagelike cell line, P388D1, by affinity chromatography on IgG-Sepharose 4B. A monoclonal clone (designated as 3A2) out of a total of 13 different antibody-secreting cell lines was found to secrete IgG1 class antibodies, which inhibited more than 70% of the binding of radio-iodinated myeloma IgG2a protein to P388D1 cells. The 3A2 Fab fragments bound specifically to P388D1 cells at 4 degrees C with a KD of  $1.9 \times 10^{-8}$  M and Bmax of  $2.9 \times 10^5$  per cell. This Fab fragment also specifically bound to Fc gamma 2a **receptor** (R)-positive T cell line (S49) with a KD of  $4.4 \times 10^{-9}$  M and a Bmax of  $1.0 \times 10^4$  but did not bind to Fc gamma 2a-negative S49 variant cell line, cyc-. The flow cytometric analysis with the use of fluorescein-isothiocyanate-tagged 3A2 F(ab')<sub>2</sub> also showed that this antibody binds to Fc gamma 2aR-positive cells, P388D1 and S49, but not to Fc gamma 2aR-negative cells, cyc-. Monomeric and heat-**aggregated**

IgG2a (13-fold molar excess) inhibited the binding of the radioiodinated 3A2 F(ab')<sub>2</sub> to P388D1 cells by 70 and 49%, respectively, whereas the inhibition by monomeric and heat-**aggregated** IgG2b was 17 and 39%, respectively; 3A2 F(ab')<sub>2</sub> (100-fold molar excess) inhibited the binding of IgG2a and IgG2b to P388D1 cells by 90 and 24%, respectively, whereas the inhibition of binding of these IgG to S49 cells was 79 and 49%, respectively. Western blotting analysis showed that 3A2 antibody recognizes a major protein (Mr = 100,000) and a minor component (Mr = 80,000) separated by SDS-PAGE of P388D1 or S49 cell lysates under nonreducing condition, whereas under reducing condition, this antibody recognized a major protein (Mr = 50,000) and two additional minor components (Mr = 40,000 and 35,000). Fc gamma 2aR may thus exist at the cell surface as a **disulfide** linked dimer of a subunit of Mr of 50,000, which could be



partially degraded during the isolation to smaller fragments of 40,000 and 35,000 Mr peptides which are still held together by interchain **disulfide** bond. (ABSTRACT TRUNCATED AT 250 WORDS)

... x 10(5) per cell. This Fab fragment also specifically bound to Fc gamma 2a **receptor** (R)-positive T cell line (S49) with a KD of 4.4 x 10(-9) M and a...

... P388D1 and S49, but not to Fc gamma 2aR-negative cells, cyc-. Monomeric and heat-**aggregated** IgG2a (13-fold molar excess) inhibited the binding of the radioiodinated 3A2 F(ab')<sub>2</sub> to P388D1 cells by 70 and 49%, respectively, whereas the inhibition by monomeric and heat-**aggregated** IgG2b was 17 and 39%, respectively; 3A2 F(ab')<sub>2</sub> (100-fold molar excess) inhibited the binding of IgG2a and IgG2b to P388D1 cells by 90 and ...

...and 35,000). Fc gamma 2aR may thus exist at the cell surface as a **disulfide** linked dimer of a subunit of Mr of 50,000, which could be partially degraded...

... of 40,000 and 35,000 Mr peptides which are still held together by interchain **disulfide** bond. (ABSTRACT TRUNCATED AT 250 WORDS)

10/3,K,AB/4 (Item 1 from file: 34)  
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci  
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09056476 Genuine Article#: 361QZ Number of References: 131

Title: Protein glucosylation and its role in protein **folding** (ABSTRACT AVAILABLE)

Author(s): Parodi AJ (REPRINT)

Corporate Source: UNIV SAN MARTIN, INST INVEST BIOTECHNOL, CASILLA CORREO 30/RA-1650 SAN MARTIN/BUENOS AIRES/ARGENTINA/ (REPRINT); FDN CAMPOMAR, INST INVEST BIOQUIM/RA-1405 BUENOS AIRES/DF/ARGENTINA/

Journal: ANNUAL REVIEW OF BIOCHEMISTRY, 2000, V69, P69-93

ISSN: 0066-4154 Publication date: 20000000

Publisher: ANNUAL REVIEWS, 4139 EL CAMINO WAY, PO BOX 10139, PALO ALTO, CA 94303-0139

Language: English Document Type: REVIEW

Abstract: An unconventional mechanism for retaining improperly **folded** glycoproteins and facilitating acquisition of their native tertiary and quaternary structures operates in the endoplasmic reticulum. Recognition of **folding** glycoproteins by two resident lectins, membrane-bound calnexin and its soluble homolog, calreticulin, is mediated by protein-linked monoglucosylated oligosaccharides. These oligosaccharides contain glucose (Glc), mannose (Man), and N-acetylglucosamine (GlcNAc) in the general form Glc(1)Man(7-9)GlcNAc(2). They are formed by glucosidase I- and II-catalyzed partial deglucosylation of the oligosaccharide transferred from dolichol diphosphate derivatives to Asn residues in nascent polypeptide chains (Glc(3)Man(9)GlcNAc(2)). Further deglucosylation of the oligosaccharides by glucosidase II liberates glycoproteins from their calnexin/calreticulin anchors. Monoglucosylated glycans are then recreated by the UDP-Glc:glycoprotein glucosyltransferase (GT), and thus recognized again by the lectins, only when linked to improperly **folded** protein moieties, as GT behaves as a sensor of glycoprotein conformations. The deglucosylation-reglucosylation cycle continues until proper **folding** is achieved. The lectin-monoglucosylated oligosaccharide interaction is one of the alternative ways by which cells retain improperly **folded** glycoproteins in the endoplasmic reticulum. Although it decreases the **folding** rate, it, increases **folding** efficiency, prevents premature glycoprotein oligomerization and degradation, and suppresses



formation of nonnative **disulfide** bonds by hindering **aggregation** and thus allowing interaction of protein moieties of **folding** glycoproteins with classical chaperones and other proteins that assist in **folding**.

Title: Protein glucosylation and its role in protein **folding**

Abstract: An unconventional mechanism for retaining improperly **folded** glycoproteins and facilitating acquisition of their native tertiary and quaternary structures operates in the endoplasmic reticulum. Recognition of **folding** glycoproteins by two resident lectins, membrane-bound calnexin and its soluble homolog, calreticulin, is mediated...

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...Identifiers--N-LINKED OLIGOSACCHARIDES; GLC-GLYCOPROTEIN GLUCOSYLTRANSFERASE; ENDOPLASMIC-RETICULUM MEMBRANE; MOLECULAR CHAPERONES CALNEXIN; VESICULAR STOMATITIS-VIRUS; **TCR** -ALPHA-PROTEINS; HISTOCOMPATIBILITY COMPLEX-MOLECULES; NICOTINIC ACETYLCHOLINE-RECEPTOR; HIGH-MANNOSE OLIGOSACCHARIDES

10/3,K,AB/5 (Item 2 from file: 34)  
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci  
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04007642 Genuine Article#: QY506 Number of References: 35

Title: CROSSRECOGNITION BY CD8 **T-CELL RECEPTOR**-ALPHA-BETA

CYTOTOXIC T-LYMPHOCYTES OF PEPTIDES IN THE SELF AND THE MYCOBACTERIAL HSP60 WHICH SHARE INTERMEDIATE SEQUENCE HOMOLOGY (Abstract Available)

Author(s): ZUGEL U; SCHOEL B; YAMAMOTO S; HENGEL H; MOREIN B; KAUFMANN SHE

Corporate Source: UNIV ULM,DEPT IMMUNOL/D-89070 ULM//GERMANY/; UNIV ULM,DEPT IMMUNOL/D-89070 ULM//GERMANY/; INST PUBL HLTH,DEPT VET PUBL HLTH/TOKYO 108//JAPAN/; UNIV HEIDELBERG,DEPT VIROL/HEIDELBERG//GERMANY/; NATL VET INST,DEPT VIROL/S-75123 UPPSALA//SWEDEN/

Journal: EUROPEAN JOURNAL OF IMMUNOLOGY, 1995, V25, N2 (FEB), P451-458  
ISSN: 0014-2980

Language: ENGLISH Document Type: ARTICLE

Abstract: Immunization of C57BL/6 mice with the mycobacterial heat shock protein (hsp) 60 in immunostimulating complexes caused the in vivo activation of autoreactive histocompatibility complex class I (H-2D(b))-restricted CD8 **T cell receptor (TcR)** alpha/beta cells. A CD8 **TcR** alpha/beta clone with specificity for the mycobacterial hsp60 peptide(499-508) was derived from this immunization, which, in addition, recognized syngeneic macrophages which had been stressed by interferon-gamma (IFN-gamma) stimulation. The stress-induced, self peptide could be extracted from IFN-gamma-stressed macrophages by acid elution, suggesting that the IFN-gamma-induced self peptide is derived from an endogenous protein. Based on our observation that lysis of stressed target cells by this cytotoxic T lymphocyte (CTL) clone was specifically inhibited by hsp60-specific antisense oligonucleotides, we used synthetic peptides representing amino acid (aa) sequences of the murine hsp60 for target cell sensitization and



identification of the relevant self peptide. Synthetic peptides representing 9-mer to 11-mer aa sequences of the murine hsp60 with asparagine in anchor position 4 or 5 as the minimal requirement for H-2D(b) binding were tested in CTL assays. The overlapping murine hsp60 peptides(162-170/171) were stimulatory at a concentration as low as 10-100 pM. Seven other peptides of the murine hsp60 required intermediate peptide concentrations of 10-100 nM for recognition by the CTL clone. Although the murine and mycobacterial hsp60 peptides recognized by this CTL clone showed only intermediate homology (3 identical and 3 similar aa), our data suggest that endogenous hsp60 itself is the source of self peptide(s) presented by IFN-gamma-stressed macrophages to the cross-reactive CTL clone with promiscuous specificity. This notion is consistent with the idea of hsp as a link between infection and autoimmunity.

Title: CROSSRECOGNITION BY CD8 T-CELL RECEPTOR-ALPHA-BETA  
CYTOTOXIC T-LYMPHOCYTES OF PEPTIDES IN THE SELF AND THE MYCOBACTERIAL  
HSP60 WHICH...

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...Research Fronts: T-LYMPHOCYTES; ANTIGEN PROCESSING)

93-0448 001 (SOLID-PHASE PEPTIDE-SYNTHESIS; AMINO PROTECTING GROUPS;  
CYCLIC DISULFIDE ANALOGS; FMOC CLEAVAGE)

93-1602 001 (PROTEIN FOLDING; MOLECULAR CHAPERONES;  
AGGREGATION OF MAMMALIAN MITOCHONDRIAL MALATE-DEHYDROGENASE  
INVITRO)

93-4806 001 (INDUCTION OF CD8+ CYTOTOXIC T-LYMPHOCYTES...

...I MHC-RESTRICTED CTL EPITOPES)

93-6931 001 (HEAT-SHOCK PROTEINS; SURFACE EXPRESSION; GAMMA-DELTA  
T-CELL RECEPTOR IN ACUTE BRUCELLA-MELITENSIS INFECTION)

93-8159 001 (65-KDA STRESS PROTEIN; MYCOBACTERIUM-TUBERCULOSIS DNA...

10/3,K,AB/6 (Item 3 from file: 34)  
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci  
(c) 2004 Inst for Sci Info. All rts. reserv.

03951631 Genuine Article#: QU616 Number of References: 26

Title: TCR-ALPHA-CD3-DELTA-EPSILON ASSOCIATION IS THE INITIAL STEP IN  
ALPHA-BETA DIMER FORMATION IN MURINE T-CELLS AND IS LIMITING IN  
IMMATURE CD4(+)CD8(+) THYMOCYTES (Abstract Available)

Author(s): KEARSE KP; ROBERTS JL; SINGER A

Corporate Source: NCI,EXPTL IMMUNOL BRANCH,BLDG 10/BETHESDA//MD/20892

Journal: IMMUNITY, 1995, V2, N4 (APR), P391-399

ISSN: 1074-7613

Language: ENGLISH Document Type: ARTICLE

Abstract: The present study has examined the molecular events leading to formation of up dimers in normal murine thymocytes and mature T cells. We demonstrate that TCR assembly proceeds by initial association of TCR alpha with CD3 delta epsilon proteins and by association of ICRP with CD3 gamma epsilon proteins to form alpha delta epsilon and beta gamma epsilon trimers; these trimers then associate to form alpha delta epsilon-beta gamma epsilon complexes, within which alpha-beta disulfide bond formation occurs. We also show that TCR-associated protein (TRAP) associates uniquely with CD3 gamma epsilon pairs and that formation of beta gamma epsilon trimers occurs subsequent to TRAP dissociation. Importantly, we document that the assembly step that is quantitatively limiting in CD4(+)CD8(+) thymocytes is the initial association of TCR alpha with CD3 delta epsilon chains, which appears necessary to protect nascent TCR



alpha proteins from accelerated degradation within the ER of immature thymocytes.

Title: **TCR-ALPHA-CD3-DELTA-EPSILON ASSOCIATION IS THE INITIAL STEP IN ALPHA-BETA DIMER FORMATION IN...**

...Abstract: formation of up dimers in normal murine thymocytes and mature T cells. We demonstrate that **TCR** assembly proceeds by initial association of **TCR** alpha with CD3 delta epsilon proteins and by association of **ICRP** with CD3 gamma epsilon...

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...Research Fronts: IMMATURE CD4+CD8+ THYMOCYTES; ADULT MURINE THYMUS; RECEPTOR OVEREXPRESSION; NEGATIVE SELECTION)

93-1602 001 (PROTEIN **FOLDING**; MOLECULAR CHAPERONES; **AGGREGATION** OF MAMMALIAN MITOCHONDRIAL MALATE-DEHYDROGENASE INVITRO)

10/3,K,AB/7 (Item 4 from file: 34)  
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci  
(c) 2004 Inst for Sci Info. All rts. reserv.

01842119 Genuine Article#: JF804 Number of References: 31

Title: TRANSIENT **AGGREGATION** OF NASCENT THYROGLOBULIN IN THE ENDOPLASMIC-RETICULUM - RELATIONSHIP TO THE MOLECULAR CHAPERONE, BIP (Abstract Available)

Author(s): KIM PS; BOLE D; ARVAN P

Corporate Source: HARVARD UNIV,BETH ISRAEL HOSP,DIV

ENDOCRINOL/BOSTON//MA/02215; HARVARD UNIV,SCH MED,CELL & DEV BIOL PROGRAM/BOSTON//MA/02115; UNIV MICHIGAN,SCH MED,DEPT BIOL CHEM/ANN ARBOR//MI/48109

Journal: JOURNAL OF CELL BIOLOGY, 1992, V118, N3 (AUG), P541-549

Language: ENGLISH Document Type: ARTICLE

Abstract: Because of its unusual length, nascent thyroglobulin (Tg) requires a long time after translocation into the endoplasmic reticulum (ER) to assume its mature tertiary structure. Thus, Tg is an ideal molecule for the study of protein **folding** and export from the ER, and is an excellent potential substrate for molecular chaperones. During the first 15 min after biosynthesis, Tg is found in transient **aggregates** with and without interchain **disulfide** bonds, which precede the formation of free monomers (and ultimately dimers) within the ER. By immunoprecipitation, newly synthesized Tg was associated with the binding protein (BiP); association was maximal at the earliest chase times. Much of the Tg released from BiP by the addition of Mg-ATP was found in **aggregates** containing interchain **disulfide** bonds; other BiP-associated Tg represented non-covalent **aggregates** and unfolded free monomers. Importantly, the immediate precursor to Tg dimer was a compact monomer which did not associate with BiP. The average stoichiometry of BiP/Tg interaction involved nearly 10 BiP molecules per Tg molecule. Cycloheximide was used to reduce the ER concentration of Tg relative to chaperones, with subsequent removal of the drug in order to rapidly restore Tg synthesis. After this treatment, nascent Tg **aggregates** were no longer detectable. The data suggest a model of **folding** of exportable proteins in which nascent polypeptides immediately upon



translocation into the ER interact with BiP. Early interaction with BiP may help in presenting nascent polypeptides to other helper molecules that catalyze **folding**, thereby preventing **aggregation** or driving **aggregate** dissolution in the ER.

Title: TRANSIENT AGGREGATION OF NASCENT THYROGLOBULIN IN THE

ENDOPLASMIC-RETICULUM - RELATIONSHIP TO THE MOLECULAR CHAPERONE, BIP

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Research Fronts: 90-1310 003 (HEAT-SHOCK PROTEINS; GAMMA-DELTA-T-CELL RECEPTOR REPERTOIRE; MAIZE MITOCHONDRIAL CHAPERONIN HSP60)  
90-7788 001 (ENDOPLASMIC-RETICULUM RETENTION SIGNAL; PROTEIN **DISULFIDE** ISOMERASE; SECRETORY PATHWAY; INVARIANT CHAIN; VESICULAR STOMATITIS-VIRUS)

10/3,K,AB/8 (Item 5 from file: 34)  
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci  
(c) 2004 Inst for Sci Info. All rts. reserv.

01820686 Genuine Article#: JE383 Number of References: 49

Title: EARLY **DISULFIDE** BOND FORMATION PREVENTS HETEROTYPIC **AGGREGATION** OF MEMBRANE-PROTEINS IN A CELL-FREE TRANSLATION SYSTEM (Abstract Available)

Author(s): YILLA M; DOYLE D; SAWYER JT

Corporate Source: SUNY BUFFALO,DEPT BIOL SCI/BUFFALO//NY/14260

Journal: JOURNAL OF CELL BIOLOGY, 1992, V118, N2 (JUL), P245-252

Language: ENGLISH Document Type: ARTICLE

Abstract: We previously demonstrated that a heterotypic complex of the two rat asialoglycoprotein receptor subunits was assembled during cell-free translation (Sawyer, J. T., and D. Doyle. 1990. Proc. Natl. Acad. Sci. USA. 87:4854-4858). We have characterized this system further by analyzing polypeptide interactions under both reducing and oxidizing translation conditions. This report shows that the complex represents a heterogeneous interaction between reduced membrane proteins rather than a specific oligomeric structure. In the reduced state membrane proteins interact in this system to form **aggregates** of diverse size and composition. The **aggregated** nascent polypeptides interact with the immunoglobulin heavy chain binding protein but this protein is not an integral component of the **aggregate**. **Aggregation** occurs via the exoplasmic domain, rather than the transmembrane domain, and the **folding** of this domain by the formation of intramolecular disulfides, prevents the interaction from occurring. Additionally, the



folded molecules containing intramolecular disulfides lack high affinity binding activity and thus appear to resemble the earliest folding intermediates seen in vivo (Olson, J. T., and M. D. Lane. 1989. FASEB (Fed. Am. Soc. Exp. Biol.) J. 3: 1618-1624). These results lead us to suggest that the formation of intramolecular disulfides during early biogenesis serves to prevent nonspecific associations between nascent polypeptides.

Title: EARLY **DISULFIDE** BOND FORMATION PREVENTS HETEROTYPIC AGGREGATION OF MEMBRANE-PROTEINS IN A CELL-FREE TRANSLATION SYSTEM

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Research Fronts: 90-1310 005 (HEAT-SHOCK PROTEINS; GAMMA-DELTA-T-CELL RECEPTOR REPERTOIRE; MAIZE MITOCHONDRIAL CHAPERONIN HSP60)  
90-1006 001 (PROTEIN **FOLDING**; SYNTHETIC PEPTIDES; HELIX COIL STABILITY-CONSTANTS FOR THE NATURALLY-OCCURRING AMINO-ACIDS)  
90-1347 001...

...CYCLASE PATHWAY; HEAT-SHOCK PROTEIN HSP70 FAMILY)  
90-7788 001 (ENDOPLASMIC-RETICULUM RETENTION SIGNAL; PROTEIN **DISULFIDE** ISOMERASE; SECRETORY PATHWAY; INVARIANT CHAIN; VESICULAR STOMATITIS-VIRUS)

10/3,K,AB/9 (Item 6 from file: 34)  
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci  
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01698903 Genuine Article#: HT965 Number of References: 40  
Title: ANALYSIS OF THE GLYCOSYLATION AND PHOSPHORYLATION OF THE ALPHA-SUBUNIT OF THE LYSOSOMAL-ENZYME, BETA-HEXOSAMINIDASE-A, BY SITE-DIRECTED MUTAGENESIS (Abstract Available)  
Author(s): WEITZ G; PROIA RL  
Corporate Source: NIDDKD, GENET & BIOCHEM BRANCH, BLDG 10, RM 9D-15/BETHESDA//MD/20892; NIDDKD, GENET & BIOCHEM BRANCH, BLDG 10, RM 9D-15/BETHESDA//MD/20892  
Journal: JOURNAL OF BIOLOGICAL CHEMISTRY, 1992, V267, N14 (MAY 15), P 10039-10044

Language: ENGLISH Document Type: ARTICLE

Abstract: The glycosylation and subsequent phosphorylation of mannose residues is a pivotal modification during the biosynthesis of lysosomal enzymes. We have identified the sites of N-linked glycosylation and oligosaccharide phosphorylation on the alpha-subunit of beta-hexosaminidase and have determined the influence of the oligosaccharides on the **folding** and transport of the enzyme. The potential glycosylation sequences, either singly or in combination, were eliminated through site-directed mutagenesis of the cDNA. By expression of the mutant cDNAs in COS-1 cells, each of the three glycosylation sites on the alpha-subunit was found to be modified by an oligosaccharide. One of the three oligosaccharides was the preferred site of phosphorylation. The absence of any individual oligosaccharide did not diminish the expression of the catalytic activity associated with the alpha-chain, implying proper **folding** and assembly of



subunits. A profound effect was observed, however, when all three oligosaccharides were absent. The unglycosylated alpha-subunit, resulting from genetic alteration of all three glycosylation sites or synthesis of the wild-type protein in the presence of tunicamycin, was catalytically inactive. It was found to be improperly **folded** into an insoluble **aggregate**, linked through inappropriate **disulfide** bonds. The unglycosylated protein was trapped in the lumen of the endoplasmic reticulum and was found in a complex with the Ig heavy chain-binding protein, BiP. The properties of the nonglycosylated, misfolded alpha-subunit were similar to some mutant alpha-subunits in Tay-Sachs disease patients. The results indicate that the oligosaccharides are essential, although not in a site-specific manner, for proper **folding** and cellular transport of the alpha-subunit.

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Research Fronts: 90-1310 001 (HEAT-SHOCK PROTEINS; GAMMA-DELTA-T-CELL **RECEPTOR** REPERTOIRE; MAIZE MITOCHONDRIAL CHAPERONIN HSP60)  
90-2081 001 (INSULIN-LIKE GROWTH FACTOR-I; CHARACTERIZATION OF...

10/3,K,AB/10 (Item 7 from file: 34)  
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci  
(c) 2004 Inst for Sci Info. All rts. reserv.

01698461 Genuine Article#: HU423 Number of References: 72  
Title: PROTEIN **FOLDING** AND PROTEIN REFOLDING (Abstract Available)  
Author(s): SECKLER R; JAENICKE R  
Corporate Source: UNIV REGENSBURG, INST BIOPHYS & PHYS BIOCHEM, UNIV STR 31/W-8400 REGENSBURG//GERMANY//; UNIV REGENSBURG, INST BIOPHYS & PHYS BIOCHEM, UNIV STR 31/W-8400 REGENSBURG//GERMANY/  
Journal: FASEB JOURNAL, 1992, V6, N8 (MAY), P2545-2552  
Language: ENGLISH Document Type: REVIEW

Abstract: The functional three-dimensional structure of proteins is determined solely by their amino acid sequences. Protein **folding** occurs spontaneously beginning with the formation of local secondary structure concomitant with a compaction of the molecule. Secondary structure elements subsequently interact to form subdomains and domains stabilized by tertiary interactions. **Disulfide** bond formation, and cis-trans isomerization of X-Pro peptide bonds, as the rate-limiting **folding** reactions, are enzymatically catalyzed during protein **folding** in the cell. Although **folding** of domains is fast enough to occur cotranslationally in vivo, such vectorial **folding** on the ribosome is not essential for attainment of the native structure of a protein. Slow steps on the pathway to the functional protein structure are docking reactions of domains,



association of subunits, or reshuffling reactions at the oligomer level. **Aggregation** as a competing side reaction is prevented, and the kinetic partition between competing polypeptide **folding** and translocation reactions is regulated by chaperone proteins binding to incompletely **folded** polypeptides.

Title: PROTEIN **FOLDING** AND PROTEIN REFOLDING

...Abstract: functional three-dimensional structure of proteins is determined solely by their amino acid sequences. Protein **folding** occurs spontaneously beginning with the formation of local secondary structure concomitant with a compaction of...

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Research Fronts: 90-1006 005 (PROTEIN **FOLDING**; SYNTHETIC PEPTIDES; HELIX COIL STABILITY-CONSTANTS FOR THE NATURALLY-OCCURRING AMINO-ACIDS)  
90-0761 001 (EXPRESSION IN ESCHERICHIA-COLI; PROTEIN **FOLDING**; RECOMBINANT GENES; ACTIVE ENZYME)  
90-1310 001 (HEAT-SHOCK PROTEINS; GAMMA-DELTA-T-CELL **RECEPTOR** REPERTOIRE; MAIZE MITOCHONDRIAL CHAPERONIN HSP60)  
90-4044 001 (PROTEIN SURFACE; MOLECULAR SHAPE; SINGLE AMINO-ACID...

10/3,K,AB/11 (Item 8 from file: 34)  
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci  
(c) 2004 Inst for Sci Info. All rts. reserv.

01667016 Genuine Article#: HR017 Number of References: 52  
Title: MISFOLDING AND **AGGREGATION** OF NEWLY SYNTHESIZED PROTEINS IN THE ENDOPLASMIC-RETICULUM (Abstract Available)

Author(s): MARQUARDT T; HELENIUS A

Corporate Source: YALE UNIV, SCH MED, DEPT CELL BIOL/NEW HAVEN//CT/06510

Journal: JOURNAL OF CELL BIOLOGY, 1992, V117, N3 (MAY), P505-513

Language: ENGLISH Document Type: ARTICLE

Abstract: As a part of our studies on the **folding** of glycoproteins in the ER, we analyzed the fate of viral glycoproteins that have misfolded either spontaneously or through inhibition of N-linked glycosylation. Newly synthesized Semliki Forest virus spike glycoproteins E1 and p62 and influenza hemagglutinin were studied in infected and transfected tissue culture cells. Misfolded proteins **aggregated** in < 1 min after release from polysomes and aberrant interchain **disulfide** bonds were formed immediately. When more than one protein was misfolded, mixed **aggregates** were generated. This indicated that the formation of complexes was nonspecific, random, and not restricted to products from single polysomes. The size of the **aggregates** varied from small oligomers to complexes of several million da tons. BiP was associated noncovalently with the **aggregates** and with some of the nonaggregated products. We conclude that **aggregation** reflects the poor solubility of incompletely **folded** polypeptide chains.

11/21/04



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Research Fronts: 90-1310 003 (HEAT-SHOCK PROTEINS; GAMMA-DELTA-T-CELL **RECEPTOR** REPERTOIRE; MAIZE MITOCHONDRIAL CHAPERONIN HSP60)  
90-0761 001 (EXPRESSION IN ESCHERICHIA-COLI; PROTEIN **FOLDING**; RECOMBINANT GENES; ACTIVE ENZYME)  
90-1006 001 (PROTEIN **FOLDING**; SYNTHETIC PEPTIDES; HELIX COIL STABILITY-CONSTANTS FOR THE NATURALLY-OCCURRING AMINO-ACIDS)  
90-6592 001...

10/3,K,AB/12 (Item 9 from file: 34)  
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci  
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01629973 Genuine Article#: HM714 Number of References: 22

Title: THE EFFECT OF GLUCOCORTICOID ON THE SUBCELLULAR-LOCALIZATION, OLIGOMERIZATION, AND PROCESSING OF MOUSE MAMMARY-TUMOR VIRUS ENVELOPE PROTEIN PRECURSOR-PR74 (Abstract Available)

Author(s): COREY JL; STALLCUP MR

Corporate Source: UNIV SO CALIF,HLTH SCI CTR,DEPT PATHOL,HMR 301,2011 ZONAL AVE/LOS ANGELES//CA/90033; UNIV SO CALIF,HLTH SCI CTR,DEPT PATHOL,HMR 301,2011 ZONAL AVE/LOS ANGELES//CA/90033; UNIV SO CALIF,HLTH SCI CTR,DEPT BIOCHEM/LOS ANGELES//CA/90033

Journal: MOLECULAR ENDOCRINOLOGY, 1992, V6, N3 (MAR), P450-458

Language: ENGLISH Document Type: ARTICLE

Abstract: Mouse lymphoma cell line W7MG1 is stably infected with mouse mammary tumor virus and produces the viral envelope glycoprotein precursor Pr74, but the mature envelope proteins gp52 and gp33, which are derived from Pr74 by posttranslational processing, are produced only when the cells are cultured with a glucocorticoid agonist. The current study demonstrated that even when W7MG1 cells are grown with hormone, the conversion of Pr74 to gp52 and gp33 is an inefficient process in this cell line. At least 2 h of exposure to glucocorticoid were required to induce the appearance of gp52 and gp33; furthermore, Pr74 labeled in the absence of hormone was not converted to gp52 and gp33 upon subsequent addition of hormone. RNA synthesis inhibitors blocked the hormonal induction of gp52 and gp33, indicating that the hormone acts by promoting the expression of a new gene(s) required for the production of gp52 and gp33, rather than by inhibiting the expression of a gene(s) that prevents processing of Pr74. Subcellular fractionation studies demonstrated that Pr74 produced in either the presence or absence of hormone was associated primarily with the ER, whereas gp52 and gp33 were found in the Golgi and plasma membrane fractions. The Pr74 molecules from W7MG1 cells grown either with or without glucocorticoid coimmunoprecipitated with BiP/GRP78 and sedimented as **aggregates** of heterogeneous size. In contrast, Pr74 from virus-producing GR3A mouse mammary tumor cells, which process



Pr74 more efficiently, sedimented as apparent monomers, dimers, and trimers. These results suggest that the failure to produce mature mouse mammary tumor virus envelope proteins gp52 and gp33 in W7MG1 cells grown without hormone is due to improper **folding** and/or oligomerization of Pr74 and, as a result, failure of Pr74 to exit from the ER.

...Abstract: W7MG1 cells grown either with or without glucocorticoid coimmunoprecipitated with BiP/GRP78 and sedimented as **aggregates** of heterogeneous size. In contrast, Pr74 from virus-producing GR3A mouse mammary tumor cells, which...

...envelope proteins gp52 and gp33 in W7MG1 cells grown without hormone is due to improper **folding** and/or oligomerization of Pr74 and, as a result, failure of Pr74 to exit from...

Research Fronts: 90-1310 001 (HEAT-SHOCK PROTEINS; GAMMA-DELTA-T-CELL **RECEPTOR** REPERTOIRE; MAIZE MITOCHONDRIAL CHAPERONIN HSP60)  
90-3517 001 (REGULATED SECRETORY PATHWAY; TRANSFECTED PC12 CELLS; RENIN

...N-LINKED OLIGOSACCHARIDES; SECRETORY PATHWAY; GOLGI MEMBRANE)  
90-7788 001 (ENDOPLASMIC-RETICULUM RETENTION SIGNAL; PROTEIN **DISULFIDE** ISOMERASE; SECRETORY PATHWAY; INVARIANT CHAIN; VESICULAR STOMATITIS-VIRUS)

10/3,K,AB/13 (Item 10 from file: 34)  
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci  
(c) 2004 Inst for Sci Info. All rts. reserv.

01611204 Genuine Article#: HL679 Number of References: 35  
Title: GROE PREVENTS THE ACCUMULATION OF EARLY **FOLDING** INTERMEDIATES OF PRE-BETA-LACTAMASE WITHOUT CHANGING THE **FOLDING** PATHWAY (Abstract Available)

Author(s): ZAHN R; PLUCKTHUN A

Corporate Source: UNIV MUNICH,GENZENTRUM,MAX PLANCK INST BIOCHEM,AM KLOPFERSPITZ/W-8033 MARTINSRIED//GERMANY/; UNIV MUNICH,GENZENTRUM,MAX PLANCK INST BIOCHEM,AM KLOPFERSPITZ/W-8033 MARTINSRIED//GERMANY/

Journal: BIOCHEMISTRY, 1992, V31, N12 (MAR 31), P3249-3255

Language: ENGLISH Document Type: ARTICLE

Abstract: In **folding** studies of pre-beta-lactamase in the presence of GroE, we investigated the pH dependence of the **folding** reaction. Two critical intermediates in the **folding** pathway were defined kinetically. I1 is an early **folding** intermediate recognized by GroE; the misfolding of I1 leads to **aggregation**, and this is prevented by GroE. A second intermediate I2 is released from GroE after ATP hydrolysis. Its pH-dependent misfolding to a nonnative form, which is not an **aggregate**, is not prevented by GroE. From these results, a model is proposed, in which the crucial role of GroE consists of allowing the change from I1 to I2 to take place in the complex. Fluorescence spectra of the pre-beta-lactamase complexed to GroE are very similar to those of the native state. The pathway of pre-beta-lactamase **folding** is not changed by GroE as evidenced by the same half-time and pH dependence of the **folding** reaction. GroE probably recognizes the signal sequence and some portion of the mature protein since mature beta-lactamase does not interact with GroE even under conditions of slow **folding**.

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GroE; the misfolding of I1 leads to **aggregation**, and this is prevented by GroE. A second intermediate I2 is released from GroE after ATP hydrolysis. Its pH-dependent misfolding to a nonnative form, which is not an **aggregate**, is not prevented by GroE. From these results, a model is proposed, in which the...

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...Identifiers--RIBULOSE BISPCHOSPHATE CARBOXYLASE; PROTEIN **DISULFIDE** -ISOMERASE; HEAT-SHOCK; ESCHERICHIA-COLI; ATP HYDROLYSIS; TRANSLOCATION; PURIFICATION; PRECURSOR; ASSOCIATION; MICROSOMES  
Research Fronts: 90-1310 009 (HEAT-SHOCK PROTEINS; GAMMA-DELTA-T -CELL **RECEPTOR** REPERTOIRE; MAIZE MITOCHONDRIAL CHAPERONIN HSP60)  
90-1006 003 (PROTEIN **FOLDING**; SYNTHETIC PEPTIDES; HELIX COIL STABILITY-CONSTANTS FOR THE NATURALLY-OCCURRING AMINO-ACIDS)  
90-0774 001...

10/3,K,AB/14 (Item 11 from file: 34)  
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci  
(c) 2004 Inst for Sci Info. All rts. reserv.

01567578 Genuine Article#: HJ944 Number of References: 30  
Title: ROLE OF ATP AND **DISULFIDE** BONDS DURING PROTEIN **FOLDING**  
IN THE ENDOPLASMIC-RETICULUM (Abstract Available) ✓  
Author(s): BRAAKMAN I; HELENIUS J; HELENIUS A  
Corporate Source: YALE UNIV,SCH MED,DEPT CELL BIOL,333 CEDAR ST/NEW HAVEN//CT/06510

Journal: NATURE, 1992, V356, N6366 (MAR 19), P260-262

Language: ENGLISH Document Type: ARTICLE

Abstract: BEING topologically equivalent to the extracellular space, the lumen of the endoplasmic reticulum (ER) provides a unique **folding** environment for newly synthesized proteins. Unlike other compartments in the cell where **folding** occurs, the ER is oxidizing and therefore can promote the formation of disulphide bonds 1. The reducing agent dithiothreitol, when added to living cells, inhibits disulphide formation with profound effects on **folding** 2. Taking advantage of this effect, we demonstrate here that **folding** of influenza haemagglutinin is energy dependent. Metabolic energy is required to support the correct **folding** and disulphide bond formation in this well characterized viral glycoprotein, to rescue misfolded proteins from disulphide-linked **aggregates**, and to maintain the oxidized protein in its **folded** and oligomerization-competent state.

Title: ROLE OF ATP AND **DISULFIDE** BONDS DURING PROTEIN **FOLDING**  
IN THE ENDOPLASMIC-RETICULUM

...Abstract: equivalent to the extracellular space, the lumen of the endoplasmic reticulum (ER) provides a unique **folding** environment for newly synthesized proteins. Unlike other compartments in the cell where **folding** occurs, the ER is oxidizing and therefore can promote the formation of disulphide bonds 1...

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maintain the oxidized protein in its **folded** and oligomerization-competent state.

Research Fronts: 90-1310 007 (HEAT-SHOCK PROTEINS; GAMMA-DELTA-T-CELL RECEPTOR REPERTOIRE; MAIZE MITOCHONDRIAL CHAPERONIN HSP60)  
90-1006 001 (PROTEIN **FOLDING**; SYNTHETIC PEPTIDES; HELIX COIL STABILITY-CONSTANTS FOR THE NATURALLY-OCCURRING AMINO-ACIDS)

10/3,K,AB/15 (Item 12 from file: 34)  
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci.  
(c) 2004 Inst for Sci Info. All rts. reserv.

01416555 Genuine Article#: GX507 Number of References: 93  
Title: SECRETION AND INVIVO **FOLDING** OF THE FAB FRAGMENT OF THE ANTIBODY MCPC603 IN ESCHERICHIA-COLI - INFLUENCE OF DISULFIDES AND CIS-PROLINES (Abstract Available)

Author(s): SKERRA A; PLUCKTHUN A

Corporate Source: UNIV MUNICH,MAX PLANCK INST BIOCHEM,GENZENTRUM,AM KLOPFERSPITZ/W-8033 MARTINSRIED//GERMANY//; UNIV MUNICH,MAX PLANCK INST BIOCHEM,GENZENTRUM,AM KLOPFERSPITZ/W-8033 MARTINSRIED//GERMANY/

Journal: PROTEIN ENGINEERING, 1991, V4, N8 (DEC), P971-979

Language: ENGLISH Document Type: ARTICLE

Abstract: Using the well-characterized antibody McPC603 as a model, we had found that the F(v) fragment can be isolated from Escherichia coli as a functional protein in good yields, whereas the amount of the correctly **folded** F(ab) fragment of the same antibody produced under identical conditions is significantly lower. In this paper, we analyse the reasons for this difference. We found that a variety of signal sequences function in the secretion of the isolated chains of the F(ab) fragment or in the co-secretion of both chains in E. coli. The low yield of functional F(ab) fragment is not caused by inefficient expression or secretion in E. coli, but by inefficient **folding** and/or assembly in the periplasm. We compared the **folding** yields for the F(v) and the F(ab) fragment in the periplasm under various conditions. Several diagnostic framework variants were constructed and their **folding** yields measured. The results show that substitutions affecting cis-proline residues and those affecting various disulphide bonds in the protein are by themselves insufficient to dramatically change the partitioning of the **folding** pathway to the native structure, and the cause must lie in a facile **aggregation** of **folding** intermediates common to all structural variants. However, all structural variants could be obtained in native form, demonstrating the general utility of the secretory expression strategy. ✓

Title: SECRETION AND INVIVO **FOLDING** OF THE FAB FRAGMENT OF THE ANTIBODY MCPC603 IN ESCHERICHIA-COLI - INFLUENCE OF DISULFIDES AND...

...Abstract: Escherichia coli as a functional protein in good yields, whereas the amount of the correctly **folded** F(ab) fragment of the same antibody produced under identical conditions is significantly lower. In...

...fragment is not caused by inefficient expression or secretion in E. coli, but by inefficient **folding** and/or assembly in the periplasm. We compared the **folding** yields for the F(v) and the F(ab) fragment in the periplasm under various conditions. Several diagnostic framework variants were constructed and their **folding** yields measured. The results show that substitutions affecting cis-proline residues and those affecting various...

...bonds in the protein are by themselves insufficient to dramatically change the partitioning of the **folding** pathway to the native structure, and the cause must lie in a facile **aggregation** of **folding** intermediates common to all structural variants.



However, all structural variants could be obtained in native...

...Identifiers--CHAINS; HIGH-LEVEL EXPRESSION; HUMAN GROWTH-HORMONE;  
HEAVY-CHAIN; NUCLEOTIDE-SEQUENCE; BETA-LACTAMASE; ALKALINE-PHOSPHATASE;  
**DISULFIDE** ISOMERASE; CRYSTAL-STRUCTURE

...Research Fronts: GENE FAMILIES; HEAVY-CHAIN VARIABLE REGIONS; SYSTEMIC  
LUPUS-ERYTHEMATOSUS; SOMATIC MUTATION)

90-1006 003 (PROTEIN **FOLDING**; SYNTHETIC PEPTIDES; HELIX COIL  
STABILITY-CONSTANTS FOR THE NATURALLY-OCCURRING AMINO-ACIDS)

90-1310 002 (HEAT-SHOCK PROTEINS; GAMMA-DELTA-T-CELL  
**RECEPTOR** REPERTOIRE; MAIZE MITOCHONDRIAL CHAPERONIN HSP60)

90-2362 002 (STA58 MAJOR ANTIGEN GENE; RHODOCOCCLUS-FASCIANS CLONING  
VECTORS; ESCHERICHIA-COLI CHROMOSOME; PRECISE IDENTIFICATION)

90-0761 001 (EXPRESSION IN ESCHERICHIA-COLI; PROTEIN **FOLDING**;  
RECOMBINANT GENES; ACTIVE ENZYME)

90-2285 001 (CARCINOEMBRYONIC ANTIGEN; EXPRESSION OF THE  
PREGNANCY-SPECIFIC BETA...

...IMMUNOSUPPRESSANT FK506; LYMPHOCYTE SIGNAL TRANSDUCTION; ACTIVATION  
ANTIGEN EXPRESSION; FLOW CYTOMETRIC ANALYSIS)

90-5686 001 (PROTEIN **DISULFIDE** ISOMERASE; PROLYL 4-HYDROXYLASE;  
RAT GASTRIC MUCIN OCCURS IN THE ROUGH ENDOPLASMIC-RETICULUM)

?



14/3,K,AB/7 (Item 1 from file: 55)  
DIALOG(R)File 55:Biosis Previews(R)  
(c) 2004 BIOSIS. All rts. reserv.

0010809895 BIOSIS NO.: 199799443955  
Recombinant soluble **TCR** (sTCR) protect T cells from suppression:  
Requirement for **aggregated**, pentameric, **disulfide**-linked  
alpha-beta heterodimers of sTCR  
AUTHOR: Paliwal V; Ptak W; Szczepanik M; Braswell E; Askenase P W  
AUTHOR ADDRESS: Yale Univ. Sch. Med., New Haven, CT, USA\*\*USA  
JOURNAL: Journal of Allergy and Clinical Immunology 99 (1 PART 2): pS302  
1997 1997  
CONFERENCE/MEETING: Joint Meeting of the American Academy of Allergy,  
Asthma and Immunology, the American Association of Immunologists and the  
Clinical Immunology Society San Francisco, California, USA February  
21-26, 1997; 19970221  
ISSN: 0091-6749  
DOCUMENT TYPE: Meeting; Meeting Abstract  
RECORD TYPE: Citation  
LANGUAGE: English

Recombinant soluble **TCR** (sTCR) protect T cells from suppression:  
Requirement for **aggregated**, pentameric, **disulfide**-linked  
alpha-beta heterodimers of sTCR  
1997  
...REGISTRY NUMBERS: **DISULFIDE**  
DESCRIPTORS:  
CHEMICALS & BIOCHEMICALS: **DISULFIDE**  
MISCELLANEOUS TERMS: ...**T CELL RECEPTOR**;



? ds

Set	Items	Description
S1	112057	TCR OR T(2N) RECEPTOR
S2	75854	DISULFIDE
S3	1810	S1 AND S2
S4	37324	DETRIMENTAL
S5	0	S3 AND S4
S6	714834	FOLD?
S7	129	S3 AND S6
S8	431131	UNSTABL? OR AGGREGAT?
S9	18	S7 AND S8
S10	15	RD (unique items)

? s s3 and s8

	1810	S3
	431131	S8
S11	48	S3 AND S8

? rd

>>>Duplicate detection is not supported for File 340.

>>>Records from unsupported files will be retained in the RD set..

...completed examining records

S12	38	RD (unique items)
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? s s12 not s6

	38	S12
	714834	S6
S13	24	S12 NOT S6

? s s13 and py<=1998

Processing

	24	S13
	33643811	PY<=1998
S14	23	S13 AND PY<=1998

? t s14/3,k,ab/1-23

14/3,K,AB/1 (Item 1 from file: 155)  
DIALOG(R)File 155:MEDLINE(R)  
(c) format only 2004 The Dialog Corp. All rts.



7977588 94043304 PMID: 7693702

In vivo control of redox potential during protein folding catalyzed by bacterial protein disulfide-isomerase (DsbA).

Wunderlich M; Glockshuber R

Institut für Biophysik und Physikalische Biochemie, Universität Regensburg, Germany.

Journal of biological chemistry (UNITED STATES) Nov 25 1993, 268 (33)

p24547-50, ISSN 0021-9258 Journal Code: 2985121R

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The formation of disulfide bonds in *Escherichia coli* is catalyzed by periplasmic protein disulfide-isomerase (DsbA). When the alpha-amylase/trypsin inhibitor from Ragi, a protein containing five intramolecular disulfide bridges, is secreted into the periplasm of *E. coli*, large amounts of misfolded inhibitor with incomplete or incorrect disulfides are accumulated. Folding of the inhibitor in the periplasm is not improved when DsbA is coexpressed and cosecreted. However, an up to 14-fold increase in correctly folded inhibitor is observed by co-expression of DsbA in conjugation with the addition of reduced glutathione to the growth medium. This peptide acts as a disulfide-shuffling reagent and can pass the outer membrane of *E. coli*. Since the influence of DsbA on the folding yield of the inhibitor is reduced in the presence of oxidized glutathione, the in vivo function of DsbA appears to be dependent on the ratio between oxidizing and **reducing** thiol equivalents in the **periplasm**. The high stability of thiol reagents against air oxidation during growth of *E. coli* allows the investigation of oxidative protein folding in vivo under controlled, thiol-dependent redox conditions.

... in vivo function of DsbA appears to be dependent on the ratio between oxidizing and **reducing** thiol equivalents in the **periplasm**. The high stability of thiol reagents against air oxidation during growth of *E. coli* allows...



8666062 95354659 PMID: 7628442

Identification and characterization of a new disulfide isomerase-like protein (DsbD) in Escherichia coli.

Missiakas D; Schwager F; Raina S

Departement de Biochimie Medicale, Centre Medical Universitaire, Switzerland.

EMBO journal (ENGLAND) Jul 17 1995, 14 (14) p3415-24, ISSN 0261-4189 Journal Code: 8208664

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Previous studies have established that DsbA and DsbC, periplasmic proteins of Escherichia coli, are two key players involved in disulfide bond formation. A search for extragenic mutations able to compensate for the lack of dsbA function in vivo led us to the identification of a new gene, designated dsbD. Lack of DsbD protein leads to some, but not all, of the phenotypic defects observed with other dsb mutations, such as hypersensitivity to dithiothreitol and to benzylpenicillin. In addition, unlike the rest of the dsb genes, dsbD is essential for bacterial growth at temperatures above 42 degrees C. Cloning of the wild-type gene and sequencing and overexpression of the protein show that dsbD is part of an operon and encodes an inner membrane protein. A 138 amino acid subdomain of the protein was purified and shown to possess an oxido-reductase activity in vitro. Expressing this subdomain in the periplasmic space helped restore the phenotypic defects associated with a dsbD null mutation. Interestingly, this domain shares 45% identity with the portion of the eukaryotic protein disulfide isomerase carrying the active site. We further show that in dsbD mutant bacteria the dithiol active sites of DsbA and DsbC proteins are mostly oxidized, as compared with wild-type bacteria. Our results argue that DsbD generates a **reducing** source in the **periplasm**, which is required for maintaining proper redox conditions. The finding that overexpression of DsbD leads to a Dsb- phenotype, very similar to that exhibited by dsbA null mutants, is in good agreement with such a model.

... mostly oxidized, as compared with wild-type bacteria. Our results argue that DsbD generates a **reducing** source in the **periplasm**,



09009637 20302544 PMID: 10841975

Periplasmic protein thiol:disulfide oxidoreductases of Escherichia coli.

Fabianek R A; Hennecke H; Thony-Meyer L

Institute of Microbiology, Swiss Federal Institute of Technology,  
ETH-Zentrum, CH-8092, Zurich, Switzerland.

FEMS microbiology reviews (NETHERLANDS) Jul 2000, 24 (3) p303-16,  
ISSN 0168-6445 Journal Code: 8902526

Document type: Journal Article; Review; Review, Tutorial

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Disulfide bond formation is part of the folding pathway for many periplasmic and outer membrane proteins that contain structural disulfide bonds. In Escherichia coli, a broad variety of periplasmic protein thiol:disulfide oxidoreductases have been identified in recent years, which substantially contribute to this pathway. Like the well-known cytoplasmic thioredoxins and glutaredoxins, these periplasmic protein thiol:disulfide oxidoreductases contain the conserved C-X-X-C motif in their active site. Most of them have a domain that displays the thioredoxin-like fold. In contrast to the cytoplasmic system, which consists exclusively of reducing proteins, the periplasmic oxidoreductases have either an oxidising, a reducing or an isomerisation activity. Apart from understanding their physiological role, it is of interest to learn how these proteins interact with their target molecules and how they are recycled as electron donors or acceptors. This review reflects the recently made efforts to elucidate the sources of oxidising and **reducing** power in the **periplasm** as well as the different properties of certain periplasmic protein thiol:disulfide oxidoreductases of E. coli.

...acceptors. This review reflects the recently made efforts to elucidate the sources of oxidising and **reducing** power in the **periplasm** as well as the different properties of certain periplasmic protein thiol:disulfide oxidoreductases of E...



? ds

Set	Items	Description
S1	5500	PERIPLASM
S2	3484505	REDUC?
S3	1270	S1 AND S2
S4	7293	NONREDUC?
S5	5	S1 AND S4
S6	3	RD (unique items)
S7	183	PERIPLASM(5N) REDUC?
S8	118	RD (unique items)
? s periplasm(5n)reducing		
	5500	PERIPLASM
	468169	REDUCING
S9	23	PERIPLASM(5N)REDUCING

? rd

>>>Duplicate detection is not supported for File 340.

>>>Records from unsupported files will be retained in the RD set.

...completed examining records

S10 11 RD (unique items)

? t s10/3,k,ab/1-11

10/3,K,AB/1 (Item 1 from file: 155)  
DIALOG(R) File 155:MEDLINE(R)



03883328 Genuine Article#: QP276 Number of References: 31  
Title: CONSTRUCTION OF A FUNCTIONAL **DISULFIDE**-STABILIZED TCR FV  
INDICATES THAT ANTIBODY AND TCR FV FRAMEWORKS ARE VERY SIMILAR IN  
STRUCTURE (Abstract Available)  
Author(s): KURUCZ I; BRINKMANN U; LEE B; SEGAL DM; REITER Y; JUNG SH;  
PASTAN I  
Corporate Source: NCI, DIV CANC BIOL DIAG & CTR, MOLEC BIOL  
LAB/BETHESDA//MD/20892; NCI, DIV CANC BIOL DIAG & CTR, EXPTL IMMUNOL  
BRANCH/BETHESDA//MD/20892  
Journal: IMMUNITY, 1995, V2, N3 (MAR), P281-287  
ISSN: 1074-7613

Language: ENGLISH Document Type: ARTICLE

Abstract: **Disulfide**-stabilized Fvs (dsFv) are recombinant Fv fragments of antibodies in which the inherently **unstable** V-H-V-L heterodimer is stabilized by an interchain **disulfide** bond engineered between structurally conserved framework positions. We now design and produce a **disulfide**-stabilized Fv of a T cell receptor. It is composed of V alpha and V beta variable domains of the 2B4 TCR stabilized by a **disulfide** bond between framework residues of the TCR Fv at a site corresponding to that used for **disulfide** stabilization of antibody Fvs. For ease of production and detection, the TCRdsFv was fused to a truncated form of Pseudomonas exotoxin (PE38). The TCR(dsFv) retains its native conformation and is much more stable than a Tcr scFv. Moreover, it is functional in biological assays. Because successful **disulfide** stabilization of the TCR Fv by the positions used for antibody Fv stabilization would not occur unless the mutated residues in TCR Fv are at positions closely similar to those in antibody Fvs, most likely within less than 1.5 Angstrom, these results provide very strong experimental evidence for the structural similarity between immunoglobulin and TCR antigen-binding variable domains.

Title: CONSTRUCTION OF A FUNCTIONAL **DISULFIDE**-STABILIZED TCR FV  
INDICATES THAT ANTIBODY AND TCR FV FRAMEWORKS ARE VERY SIMILAR IN  
STRUCTURE  
, 1995

1/21/04



A GENERAL-METHOD FOR FACILITATING HETERODIMERIC PAIRING BETWEEN 2  
PROTEINS - APPLICATION TO EXPRESSION OF ALPHA-T-CELL AND BETA-T-  
CELL RECEPTOR EXTRACELLULAR SEGMENTS (Abstract Available)

Author(s): CHANG HC; BAO ZZ; YAO Y; TSE AGD; GOYARTS EC; MADSEN M; KAWASAKI  
E; BRAUER PP; SACCHETTINI JC; NATHENSON SG; REINHERZ EL

Corporate Source: HARVARD UNIV, SCH MED, DANA FARBER CANC INST, IMMUNOBIOLOG  
LAB, 44 BINNEY ST/BOSTON//MA/02115; HARVARD UNIV, SCH MED, DEPT  
MED/BOSTON//MA/02115; HARVARD UNIV, SCH MED, DEPT PATHOL/BOSTON//MA/02115  
; YESHIVA UNIV ALBERT EINSTEIN COLL MED, DEPT MICROBIOL &  
IMMUNOL/BRONX//NY/10461; YESHIVA UNIV ALBERT EINSTEIN COLL MED, DEPT  
BIOCHEM/BRONX//NY/10461; YESHIVA UNIV ALBERT EINSTEIN COLL MED, DEPT  
CELLBIOL/BRONX//NY/10461; PROCEPT INC/CAMBRIDGE//MA/02139

Journal: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED  
STATES OF AMERICA, 1994, V91, N24 (NOV 22), P11408-11412

ISSN: 0027-8424

Language: ENGLISH Document Type: ARTICLE

Abstract: Generation of **soluble T-cell receptor**

(TCR) molecules by a variety of genetic engineering methods has been hampered by inefficient pairing of alpha and beta subunits in the absence of their respective transmembrane regions and associated CD3 components. To overcome this obstacle, we have added 30-amino acid-long segments to the carboxyl termini of alpha and beta extracellular domains via a cleavable flexible linker. These peptide segments (BASE-p1 for alpha and ACID-p1 for beta) have been previously shown to selectively associate to form a stable heterodimeric coiled coil termed a leucine **zipper**. Homodimeric structures are not permitted due to electrostatic repulsion among amino acid side chains. Expression of a representative TCR-leucine **zipper** fusion protein in a baculovirus expression system results in production of alpha beta TCR heterodimer at 0.6-1.4 mg/liter. This yield is 5- to 10-fold greater than that of the TCR expressed in the absence of the synthetic leucine **zipper** sequence. The structure of the TCR component of the fusion heterodimer was judged to be native when probed with a panel of 17 mAbs specific for alpha and beta constant and variable domains. A mAb specific for the isolated BASE-p1/ACID-p1 coiled coil was also generated and shown to react with the TCR fusion protein. The above technology should be broadly useful in the efficient production and purification of TCRs as well as other heterodimeric proteins.

...Title: FACILITATING HETERODIMERIC PAIRING BETWEEN 2 PROTEINS -  
APPLICATION TO EXPRESSION OF ALPHA-T-CELL AND BETA-T-CELL  
RECEPTOR EXTRACELLULAR SEGMENTS  
, 1994

Abstract: Generation of **soluble T-cell receptor**

(TCR) molecules by a variety of genetic engineering methods has been hampered by inefficient pairing...

...previously shown to selectively associate to form a stable heterodimeric coiled coil termed a leucine **zipper**. Homodimeric structures are not permitted due to electrostatic repulsion among amino acid side chains. Expression of a representative TCR-leucine **zipper** fusion protein in a baculovirus expression system results in production of alpha beta TCR heterodimer...

...fold greater than that of the TCR expressed in the absence of the synthetic leucine **zipper** sequence. The structure of the TCR component of the fusion heterodimer was judged to be...



01664046 Genuine Article#: HR186 Number of References: 23  
Title: SPECIFIC LOW-AFFINITY RECOGNITION OF MAJOR HISTOCOMPATIBILITY  
COMPLEX PLUS PEPTIDE BY **SOLUBLE T-CELL RECEPTOR**

(Abstract Available)

Author(s): WEBER S; TRAUNECKER A; OLIVERI F; GERHARD W; KARJALAINEN K


Corporate Source: BASEL INST IMMUNOL, GRENZACHERSTR 487/CH-4005

BASEL//SWITZERLAND//; WISTAR INST/PHILADELPHIA//PA/19104

Journal: NATURE, 1992, V356, N6372 (APR 30), P793-796

Language: ENGLISH Document Type: ARTICLE

Abstract: THE T-cell receptor is necessary and sufficient for recognition of peptides presented by major histocompatibility complex molecules 1,2. Other adhesion molecules, like CD4 or CD8, play an auxiliary role in antigen recognition by T cells 3,4. Here we analyse **T-cell receptor (TCR) binding** using a **soluble** rather than a cell-bound receptor molecule. A TCR-immunoglobulin chimaera is constructed with the variable and the first constant regions of both the TCR alpha- and beta-chains linked to the immunoglobulin light-chain constant regions. This soluble TCR is expressed, assembled and secreted as an alpha-beta-heterodimer by a myeloma cell line transfected with the recombinant genes. Furthermore, the soluble TCR is biologically active: it specifically inhibits antigen-dependent activation of the relevant T-cell clones and thus discriminates between proper and irrelevant peptides presented by major histocompatibility complex molecules.



Title: SPECIFIC LOW-AFFINITY RECOGNITION OF MAJOR HISTOCOMPATIBILITY  
COMPLEX PLUS PEPTIDE BY **SOLUBLE T-CELL RECEPTOR**

, 1992

...Abstract: play an auxiliary role in antigen recognition by T cells 3,4.

Here we analyse **T-cell receptor (TCR) binding**

using a **soluble** rather than a cell-bound receptor molecule. A

TCR-immunoglobulin chimaera is constructed with the...

Research Fronts: 90-0712 001 (T-CELL RECEPTOR; CLONAL DELETION;  
TRANSGENIC MICE; PERIPHERAL MECHANISMS INDUCING TISSUE TOLERANCE;  
**MHC CLASS-II MOLECULES**)

90-1517 001 (T-CELL RECEPTOR; CLASS-I MOLECUL



11193828 98070386 PMID: 9405420

Conformational integrity and **ligand** binding properties of a single chain T-cell receptor expressed in *Escherichia coli*.

Khandekar S S; Bettencourt B M; Wyss D F; Naylor J W; Brauer P P; Huestis K; Dwyer D S; Proffy A T; Osburne M S; Banerji J; Jones B

Procept, Inc., Cambridge, Massachusetts 02139, USA.

Journal of biological chemistry (UNITED STATES) Dec 19 1997, 272

(51) p32190-7, ISSN 0021-9258 Journal Code: 2985121R

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

We recently showed that a **soluble**, heterodimeric murine D10 T **-cell receptor** (TCR) (Valpha2Calpha, Vbeta8.2Cbeta) expressed in insect cells binds both Vbeta8.2-specific bacterial superantigen staphylococcal enterotoxin C2 (SEC2) and a soluble, heterodimeric major histocompatibility complex class II I-Ak.conalbumin peptide complex with a low micromolar affinity. To define further the structural requirements for the TCR/**ligand** interactions, we have produced in *Escherichia coli* a soluble, functional D10 single chain (sc) TCR molecule in which the Valpha and Vbeta domains are connected by a flexible peptide linker. Purified and refolded D10 scTCR bound to SEC2 and murine major histocompatibility complex class II I-Ak.conalbumin peptide complex with thermodynamic and kinetic binding constants similar to those measured for the baculovirus-derived heterodimeric D10 TCR suggesting that neither the TCR constant domains nor potential N- or O-linked carbohydrate moieties are necessary for **ligand** recognition and for expression and proper folding of the D10 scTCR. Purified D10 scTCR remained soluble at concentrations up to 1 mM. Circular dichroism and NMR spectroscopy indicated that D10 scTCR is stabilized predominantly by beta-sheet secondary structure, consistent with its native-like conformation. Because of its limited size, high solubility, and structural integrity, purified D10 scTCR appears to be suitable for structural studies by multidimensional NMR spectroscopy.



10483985 96293497 PMID: 8692966

Covalent assembly of a **soluble T cell receptor**  
-peptide-major histocompatibility class I complex.

Gregoire C; Lin S Y; Mazza G; Rebai N; Luescher I F; Malissen B  
Centre d'Immunologic, Institut National de la Sante et de la Recherche  
Medicale, Marseille, France.

Proceedings of the National Academy of Sciences of the United States of  
America (UNITED STATES) Jul 9 1996, 93 (14) p7184-9, ISSN

0027-8424 Journal Code: 7505876

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

We used stepwise photochemical cross-linking for specifically assembling soluble and covalent complexes made of a T-cell antigen receptor (TCR) and a class I molecule of the major histocompatibility complex (MHC) bound to an antigenic peptide. For that purpose, we have produced in myeloma cells a single-chain Fv construct of a TCR specific for a photoreactive H-2Kd-peptide complex. Photochemical cross-linking of this TCR single-chain Fv with a soluble form of the photoreactive H-2Kd-peptide **ligand** resulted in the formation of a ternary covalent complex. We have characterized the soluble ternary complex and showed that it reacted with antibodies specific for epitopes located either on the native TCR or on the Kd molecules. By preventing the fast dissociation kinetics observed with most T cell receptors, this approach provides a means of preparing soluble TCR-peptide-MHC complexes on large-scale levels.

Covalent assembly of a **soluble T cell receptor**  
-peptide-major histocompatibility class I complex.

Jul 9 1996,

... this TCR single-chain Fv with a soluble form of the photoreactive H-2Kd-peptide **ligand** resulted in the formation of a ternary covalent



06222397 Genuine Article#: YC950 Number of References: 58

Title: Analysis of the expression of peptide-major histocompatibility complexes using high affinity soluble divalent T cell receptors (ABSTRACT AVAILABLE)

Author(s): OHerrin SM; Lebowitz MS (REPRINT) ; Bieler JG; alRamadi BK; Utz U; Bothwell ALM; Schneck JP

Corporate Source: JOHNS HOPKINS MED INST,DEPT PATHOL, 720 RUTLAND AVE, 664 G ROSS BLDG/BALTIMORE//MD/21205 (REPRINT); JOHNS HOPKINS UNIV,DEPT PATHOL & MED, DIV IMMUNOPATHOL/BALTIMORE//MD/21205; YALE UNIV,SCH MED, IMMUNOBIOLOG SECT/NEW HAVEN//CT/06520; INST RECH CLIN MONTREAL,IMMUNOL LAB/MONTREAL/PQ H2W 1R7/CANADA/

Journal: JOURNAL OF EXPERIMENTAL MEDICINE, 1997, V186, N8 (OCT 20), P 1333-1345

ISSN: 0022-1007 Publication date: 19971020

Publisher: ROCKEFELLER UNIV PRESS, 1114 FIRST AVE, 4TH FL, NEW YORK, NY 10021

Language: English Document Type: ARTICLE

Abstract: Understanding the regulation of cell surface expression of specific peptide-major histocompatibility complex (MHC) complexes is hindered by the lack of direct quantitative analyses of specific peptide-MHC complexes. We have developed a direct quantitative biochemical approach by engineering soluble divalent T cell receptor analogues (TCR-Ig) that have high affinity for their cognate peptide-MHC ligands. The generality of this approach was demonstrated by specific staining of peptide-pulsed cells with two different TCR-Ig complexes: one specific for the murine alloantigen 2C, and one specific for a viral peptide from human T lymphocyte virus-1 presented by human histocompatibility leukocyte antigens-A2. Further, using 2C TCR-Ig, a more detailed analysis of the interaction with cognate peptide-MHC complexes revealed several interesting findings. Soluble divalent 2C TCR-Ig detected significant changes in the level of specific antigenic-peptide MHC cell surface expression in cells treated with gamma-interferon (gamma-IFN). Interestingly, the effects of gamma-IFN on expression of specific peptide-MHC complexes recognized by 2C TCR-Ig were distinct from its effects on total H-2 L-d expression; thus, lower doses of gamma-IFN were required to increase expression of cell surface class I MHC complexes than were required for upregulation of expression of specific peptide-MHC complexes. Analysis of the binding of 2C TCR-Ig for specific peptide-MHC ligands unexpectedly revealed that the affinity of the 2C TCR-IS for the naturally occurring alloreactive, putatively, negatively selecting, complex, DEV-8-H-2 K-bm3, is very low, weaker than 71  $\mu$  M. The affinity of the 2C TCR for the other naturally occurring, negatively selecting, alloreactive complex, p2Ca-H-2 L-d, is similar to 1000-fold higher. Thus, negatively selecting peptide-MHC complexes do not necessarily have intrinsically high affinity for cognate TCR. These results, uniquely revealed by this analysis, indicate the importance of using high affinity biologically relevant cognates, such as soluble divalent TCR, in furthering our understanding of immune responses.

, 1997

...Abstract: of specific peptide-MHC complexes. We have developed a direct quantitative biochemical approach by engineering soluble divalent T cell receptor analogues (TCR-Ig) that have high affinity for their cognate peptide-MHC ligands. The generality...

...Research Fronts: MAJOR INFECTION IN MICE; TH1 CELLS; CYTOKINE THERAPY)

95-0691 001 (T-CELL COSTIMULATION; CD40 LIGAND EXPRESSION; INDUCTION OF ANERGY; ANTIGEN RECEPTORS; IMMUNOGLOBULIN SUPERFAMILY; CTLA4IG IN-VITRO)

95-1368 001 (POSITIVE...



DIALOG(R)File 34:SciSearch(R) Cited Ref Sci  
(c) 2004 Inst for Sci Info. All rts. reserv.



7/3,K,AB/6 (Item 2 from file: 55)  
DIALOG(R)File 55:Biosis Previews(R)  
(c) 2004 BIOSIS. All rts. reserv.

0010804336 BIOSIS NO.: 199799438396  
Expression of **soluble** T-cell: Leucine **zipper** fusions  
AUTHOR: Jakobsen Bent (Reprint); Gao Fu; Wilcox Ben; Wyer Jessica R; Vessey  
S J Rupert; O'Callaghan Christopher; Bell John I  
AUTHOR ADDRESS: Mol. Immunol. Group, Inst. Mol. Med., Oxford Univ., John  
Radcliffe Hosp., Headington, Oxford OX9 3DU, UK\*\*UK  
JOURNAL: Immunotechnology (Amsterdam) 2 (4): p309 1996 **1996**  
CONFERENCE/MEETING: 1996 Keystone Meeting on Exploring and Exploiting  
Antibody and Ig Superfamily Combining Sites Taos, New Mexico, USA  
February 22-28, 1996; 19960222  
ISSN: 1380-2933  
DOCUMENT TYPE: Meeting; Meeting Abstract  
RECORD TYPE: Citation  
LANGUAGE: English

Expression of **soluble** T-cell: Leucine **zipper** fusions  
**1996**

DESCRIPTORS:

MISCELLANEOUS TERMS: ...LEUCINE ZIPPER; ...



SYSTEM:OS - DIALOG OneSearch

File 155:MEDLINE(R) 1966-2004/Jan W2

(c) format only 2004 The Dialog Corp.

\*File 155: Medline is updating again (12-22-2003).

Please see HELP NEWS 154, for details.

File 55:Biosis Previews(R) 1993-2004/Jan W2

(c) 2004 BIOSIS

File 34:SciSearch(R) Cited Ref Sci 1990-2004/Jan W2

(c) 2004 Inst for Sci Info

\*File 34: New prices as of 1/1/2004 per Information Provider request. See HELP RATES 34.

File 434:SciSearch(R) Cited Ref Sci 1974-1989/Dec

(c) 1998 Inst for Sci Info

\*File 434: New prices as of 1/1/2004 per Information Provider request. See HELP RATES434.

File 340:CLAIMS(R)/US Patent 1950-03/Jan 15

(c) 2004 IFI/CLAIMS(R)

\*File 340: Enter HELP NEWS340 & HELP ALERTS340 for search, display & Alert information.

Set Items Description

--- -----

? s t(w)cell(w)receptor??

Processing

4708001 T

4968853 CELL

2058509 RECEPTOR??

S1 77197 T(W)CELL(W)RECEPTOR??

? s zipper

S2 19632 ZIPPER

? s s1 and s2

77197 S1

19632 S2

S3 185 S1 AND S2

? s s3 and py<=1998

Processing

185 S3

33643774 PY<=1998

S4 158 S3 AND PY<=1998

? s soluble

S5 418046 SOLUBLE

? s s4 and s5

158 S4

418046 S5

S6 26 S4 AND S5

? rd

>>>Duplicate detection is not supported for File 340.

>>>Records from unsupported files will be retained in the RD set.

...completed examining records

S7 19 RD (unique items)

? t s7/3,k,ab/1-19

7/3,K,AB/1 (Item 1 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

(c) format only 2004 The Dialog Corp. All rts. reserv.

11534993 98426238 PMID: 9751750

Expression and crystallization of the complex of HLA-DR2 (DRA, DRB1\*1501) and an immunodominant peptide of human myelin basic protein.

Gauthier L; Smith K J; Pyrdol J; Kalandadze A; Strominger J L; Wiley D C; Wucherpfennig K W

Department of Cancer Immunology & AIDS, Dana-Farber Cancer Institute,



Boston, MA 02115, USA.

Proceedings of the National Academy of Sciences of the United States of America (UNITED STATES) Sep 29 1998, 95 (20) p11828-33, ISSN 0027-8424 Journal Code: 7505876

Contract/Grant No.: AI 42316; AI; NIAID; HD-17461; HD; NICHD

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

HLA-DR2 is associated with susceptibility to multiple sclerosis (MS). A peptide from human myelin basic protein (MBP, residues 85-99) was previously found to bind to purified HLA-DR2 (DRA, DRB1\*1501) and to be recognized by human MBP-specific T cell clones. Soluble HLA-DR2 was expressed in the baculovirus system by replacing the hydrophobic transmembrane regions and cytoplasmic segments of DRalpha and DRbeta with leucine zipper dimerization domains from the transcription factors Fos and Jun. In the



?  
PLEASE ENTER A COMMAND OR BE LOGGED OFF IN 5 MINUTES  
? ds

Set	Items	Description
S1	77197	T(W) CELL(W) RECEPTOR??
S2	19632	ZIPPER
S3	185	S1 AND S2
S4	158	S3 AND PY<=1998
S5	418046	SOLUBLE
S6	26	S4 AND S5
S7	19	RD (unique items)

? s soluble(5n) (T(w) cell(w) receptor)

Processing

418046	SOLUBLE
4708001	T
4968853	CELL
1632257	RECEPTOR
S8	245 SOLUBLE(5N) (T(W) CELL(W) RECEPTOR)

? s ligand

S9	356655	LIGAND
----	--------	--------

? s s8 and s9

245	S8
356655	S9
S10	47 S8 AND S9

? rd

>>>Duplicate detection is not supported for File 340.

>>>Records from unsupported files will be retained in the RD set.  
...completed examining records

S11	29	RD (unique items)
-----	----	-------------------

? s s11 and py<=1998

Processing

29	S11
33643774	PY<=1998
S12	19 S11 AND PY<=1998

? t s12/3,k,ab/1-19

12/3,K,AB/1 (Item 1 from file: 155)  
DIALOG(R) File 155:MEDLINE(R)  
(c) format only 2004 The Dialog Corp. All rts. reserv.

11548713 98440384 PMID: 9767439

Bolus injection of aqueous antigen leads to a high density of T-cell-receptor ligand in the spleen, transient T-cell activation and anergy induction.

Switzer S K; Wallner B P; Briner T J; Sunshine G H; Bourque C R; Luqman M  
ImmuLogic Pharmaceutical Corporation, Waltham, MA, USA.

Immunology (ENGLAND) Aug 1998, 94 (4) p513-22, ISSN 0019-2805

Journal Code: 0374672

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Co



18/3,K,AB/29 (Item 16 from file: 34)  
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci  
(c) 2004 Inst for Sci Info. All rts. reserv.

01583025 Genuine Article#: HJ870 Number of References: 0

Title: STUDYING COMPLEX CELL CELL-**INTERACTIONS** VIA **SOLUBLE**  
MOLECULES - **T-CELL RECEPTOR**-MEDIATED RECOGNITION OF  
PEPTIDE **MHC**

Author(s): DAVIS MM

Corporate Source: STANFORD UNIV,MED CTR,SCH MED,HOWARD HUGHES  
MEDINST/STANFORD//CA/94305; STANFORD UNIV,MED CTR,SCH MED,DEPT  
MICROBIOL & IMMUNOL/STANFORD//CA/94305

Journal: CLINICAL CHEMISTRY, 1992, V38, N3 (MAR), P450-451

Language: ENGLISH Document Type: ARTICLE

Title: STUDYING COMPLEX CELL CELL-**INTERACTIONS** VIA **SOLUBLE**  
MOLECULES - **T-CELL RECEPTOR**-MEDIATED RECOGNITION OF  
PEPTIDE **MHC**

, 1992

?



03421100 Genuine Article#: PE388 Number of References: 37

Title: BLINDING OF A **SOLUBLE ALPHA-BETA-T-CELL**

**RECEPTOR TO SUPERANTIGEN MAJOR HISTOCOMPATIBILITY COMPLEX LIGANDS**

(Abstract Available)

Author(s): KAPPLER J; WHITE J; KOZONO H; CLEMENTS J; MARRACK P

Corporate Source: NATL JEWISH CTR IMMUNOL & RESP MED, HOWARD HUGHES MED  
INST, DEPT MED, DIV BASIC IMMUNOL/DENVER//CO/80206; UNIV COLORADO, SCH  
MED, DEPT IMMUNOL/DENVER//CO/80206; UNIV COLORADO, SCH MED, DEPT  
MED/DENVER//CO/80206; UNIV COLORADO, SCH MED, DEPT BIOCHEM BIOPHYS &  
GENET/DENVER//CO/80206

Journal: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED  
STATES OF AMERICA, 1994, V91, N18 (AUG 30), P8462-8466

ISSN: 0027-8424

Language: ENGLISH Document Type: ARTICLE

Abstract: The genes for the alpha and beta chains of a murine T-cell  
receptor were truncated just prior to the portions encoding the  
transmembrane regions and introduced into baculovirus by recombination.  
Insect cells infected with the virus secreted a soluble form of the  
receptor that could be purified to homogeneity. This soluble receptor  
reacted with a set of six monoclonal antibodies originally raised to  
different epitopes on the natural transmembrane-region-containing  
receptor and bound with appropriate specificity to a cell surface  
complex of the human major histocompatibility complex class II molecule  
DR1 with the bacterial superantigen staphylococcal enterotoxin B.

Title: BLINDING OF A **SOLUBLE ALPHA-BETA-T-CELL**

**RECEPTOR TO SUPERANTIGEN MAJOR HISTOCOMPATIBILITY COMPLEX LIGANDS**

, 1994

...Identifiers--STAPHYLOCOCCAL ENTEROTOXIN-B; MONOCLONAL-ANTIBODY; ANTIGEN  
RECEPTOR; V-BETA; PEPTIDE; RECOGNITION; **BINDING**; HETERODIMERS;  
MOLECULES; HLA-DR1

...Research Fronts: HUMAN T-CELL RECEPTOR; CLONAL DELETION; NEGATIVE  
SELECTION; INVIVO TOLERANCE; MLS ANTIGENS)

92-1511 002 (**MHC CLASS-II MOLECULES**; T-CELL RECEPTOR V-BETA GENE  
USAGE; **INTERACTION OF ANTIGENIC PEPTIDES**)

92-2667 002 (**MAJOR HISTOCOMPATIBILITY COMPLEX CLASS-I MOLECULES**;  
ANTIGEN PRESENTATION; CELLULAR...

18/3,K,AB/22 (Item 9 from file: 34)

DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

(c) 2004 Inst for Sci Info. All rts. reserv.

03089190 Genuine Article#: NB718 Number of References: 57

Title: EARLY DEGENERATE SELECTION OF THYMOCYTES BY CLASS-I MAJOR

HISTOCOMPATIBILITY COMPLEX (Abstract Available)

Author(s): GRASSI F; BARBIER E; CAZENAVE PA

Corporate Source: INST PASTEUR, DEPT IMMUNOL, CNRS, UA 359, UNITE IMMUNOCHIM  
ANALYT, 25 RUE DR ROUX/F-75724 PARIS 15//FRANCE/; UNIV PARIS  
06/PARIS//FRANCE/

Journal: EUROPEAN JOURNAL OF IMMUNOLOGY, 1994, V24, N3 (MAR), P  
627-634

ISSN: 0014-2980

Language: ENGLISH Document Type: ARTICLE



03457708 Genuine Article#: PG521 Number of References: 59

Title: **BINDING OF SOLUBLE NATURAL LIGANDS TO A SOLUBLE HUMAN**

**T-CELL RECEPTOR FRAGMENT PRODUCED IN ESCHERICHIA-COLI**

(Abstract Available)

Author(s): HILYARD KL; REYBURN H; CHUNG S; BELL JI; STROMINGER JL

Corporate Source: ROCHE PROD LTD, BROADWATER RD/WELWYN GARDEN CIT AL7

3AY/HERTS/ENGLAND/; HARVARD UNIV, DEPT BIOCHEM & MOLEC

BIOL/CAMBRIDGE//MA/02138; JOHN RADCLIFFE HOSP, INST MOLEC MED, MOLEC

IMMUNOL GRP/OXFORD OX3 9DU//ENGLAND/

Journal: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED

STATES OF AMERICA, **1994**, V91, N19 (SEP 13), P9057-9061

ISSN: 0027-8424

Language: ENGLISH Document Type: ARTICLE

Abstract: An Escherichia coli expression system has been developed to produce milligram quantities- of the variable domains of a human T-cell receptor from a cytotoxic T cell that recognizes the HLA-A2-influenza matrix peptide complex as a single polypeptide chain. The recombinant protein was purified by metal-chelate chromatography and then refolded in a redox buffer system. The refolded protein was shown to directly **bind** both Staphylococcus aureus enterotoxin B and the major histocompatibility complex protein-peptide complex using a BIAcore biosensor. Thus this preparation of a single-chain, variable-domain, T cell receptor fragment can **bind** both of its natural ligands and some of it is therefore a functional fragment of the receptor molecule.

Title: **BINDING OF SOLUBLE NATURAL LIGANDS TO A SOLUBLE HUMAN**

**T-CELL RECEPTOR FRAGMENT PRODUCED IN ESCHERICHIA-COLI**

, **1994**

...Abstract: and then refolded in a redox buffer system. The refolded protein was shown to directly **bind** both Staphylococcus aureus enterotoxin B and the major histocompatibility complex protein-peptide complex using...



011180882 BIOSIS NO.: 199799814942

Analysis of the expression of peptide-major histocompatibility complexes using high affinity soluble divalent T cell receptors

AUTHOR: O'Herrin Sean M; Lebowitz Michael S (Reprint); Bieler Joan G;

Al-Ramadi Basel K; Utz Ursula; Bothwell Alfred L M; Schneck Jonathan P  
AUTHOR ADDRESS: Johns Hopkins Med. Inst., Dep. Pathol., 720 Rutland Ave.,  
664 G Ross Build., Baltimore, MD 21205, USA\*\*USA

JOURNAL: Journal of Experimental Medicine 186 (8): p1333-1345 1997

1997

ISSN: 0022-1007

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Understanding the regulation of cell surface expression of specific peptide-major histocompatibility complex (MHC) complexes is hindered by the lack of direct quantitative analyses of specific peptide-MHC complexes. We have developed a direct quantitative biochemical approach by engineering soluble divalent T cell receptor analogues (TCR-Ig) that have high affinity for their cognate peptide-MHC ligands. The generality of this approach was demonstrated by specific staining of peptide-pulsed cells with two different TCR-Ig complexes: one specific for the murine alloantigen 2C, and one specific for a viral peptide from human T lymphocyte virus-1 presented by human histocompatibility leukocyte antigens-A2. Further, using 2C TCR-Ig, a more detailed analysis of the interaction with cognate peptide-MHC complexes revealed several interesting findings. Soluble divalent 2C TCR-Ig detected significant changes in the level of specific antigenic-peptide MHC cell surface expression in cells treated with gamma-interferon (gamma-IFN). Interestingly, the effects of gamma-IFN on expression of specific peptide-MHC complexes recognized by 2C TCR-Ig were distinct from its effects on total H-2 L-d expression; thus, lower doses of gamma-IFN were required to increase expression of cell surface class I MHC complexes than were required for upregulation of expression of specific peptide-MHC complexes. Analysis of the binding of 2C TCR-Ig for specific peptide-MHC ligands unexpectedly revealed that the affinity of the 2C TCR-Ig for the naturally occurring alloreactive, putatively, negatively selecting, complex, dEV-8-H-2 K-bm3, is very low, weaker than 71  $\mu$ M. The affinity of the 2C TCR for the other naturally occurring, negatively selecting, alloreactive complex, p2Ca-H-2L-d, is approx 1000-fold higher. Thus, negatively selecting peptide-MHC complexes do not necessarily have intrinsically high affinity for cognate TCR. These results, uniquely revealed by this analysis, indicate the importance of using high affinity biologically relevant cognates, such as soluble divalent TCR, in furthering our understanding of immune responses.

1997

ABSTRACT: Understanding the regulation of cell surface expression of specific peptide-major histocompatibility complex (MHC) complexes is hindered by the lack of direct quantitative analyses of specific peptide-MHC complexes. We have developed a direct quantitative biochemical approach by engineering soluble divalent T cell receptor analogues (TCR-Ig) that have high affinity for their cognate peptide-MHC ligands. The generality of this approach was demonstrated by specific staining of peptide-pulsed cells...

...histocompatibility leukocyte antigens-A2. Further, using 2C TCR-Ig, a more detailed analysis of the interaction with cognate peptide-MHC complexes revealed several interesting findings. Soluble divalent 2C TCR-Ig detected significant changes in the level of specific



antigenic-peptide **MHC** cell surface expression in cells treated with gamma-interferon (gamma-IFN). Interestingly, the effects of gamma-IFN on expression of specific peptide-**MHC** complexes recognized by 2C TCR-Ig were distinct from its effects on total H-2...

...lower doses of gamma-IFN were required to increase expression of cell surface class I **MHC** complexes than were required for upregulation of expression of specific peptide-**MHC** complexes. Analysis of the **binding** of 2C TCR-Ig for specific peptide-**MHC** ligands unexpectedly revealed that the affinity of the 2C TCR-Ig for the naturally occurring...

...alloreactive complex, p2Ca-H-2L-d, is approx 1000-fold higher. Thus, negatively selecting peptide-**MHC** complexes do not necessarily have intrinsically high affinity for cognate TCR. These results, uniquely revealed...

18/3,K,AB/14 (Item 1 from file: 34)  
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci  
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18/3,K,AB/9 (Item 9 from file: 155)  
DIALOG(R)File 155:MEDLINE(R)  
(c) format only 2004 The Dialog Corp. All rts. reserv.

07767002 93222495 PMID: 8467090

**Interaction of the T cell receptor with bacterial superantigens.**

Gascoigne N R

Department of Immunology, Scripps Research Institute, La Jolla, CA 92037.

Seminars in immunology (UNITED STATES) Feb 1993, 5 (1) p13-21,

ISSN 1044-5323 Journal Code: 9009458

Contract/Grant No.: GM-39476; GM; NIGMS; GM-46134; GM; NIGMS

Document type: Journal Article; Review; Review, Tutorial

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Bacterial toxin superantigens **bind** to MHC class II molecules and activate a large proportion of T cells through a direct **interaction** with the T cell receptor (TCR). The toxin: TCR **interaction** involves specific recognition between the beta-chain variable region and the toxin. Although a complete alpha beta T cell receptor is required for activation of T cells, studies using purified **soluble T cell receptor** beta-chain have shown that it alone is sufficient for **binding** the toxin: class II complex. The regions of V beta and enterotoxin involved in the recognition have been determined.

**Interaction of the T cell receptor with bacterial superantigens.**

Feb 1993,

Bacterial toxin superantigens **bind** to MHC class II molecules and activate a large proportion of T cells through a direct **interaction** with the T cell receptor (TCR). The toxin: TCR **interaction** involves specific recognition between the beta-chain variable region a



73608 94239529 PMID: 8183371

Binary and ternary complexes between T-cell receptor, class II **MHC** and superantigen in vitro.

Seth A; Stern L J; Ottenhoff T H; Engel I; Owen M J; Lamb J R; Klausner R D; Wiley D C

Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts 02138.

Nature (ENGLAND) May 26 1994, 369 (6478) p324-7, ISSN

0028-0836 Journal Code: 0410462

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Superantigens are proteins that in association with class II major histocompatibility complex (**MHC**)-bearing cells can stimulate virtually all T cells that express particular classes of the variable beta-domains of the T-cell receptor (TCR). This mechanism of T-cell activation circumvents the usual requirement for peptide-specific **MHC** recognition. Staphylococcus aureus enterotoxin B (SEB) is a bacterial superantigen that causes food poisoning and shock. We have characterized the tertiary complex of SEB, a **soluble T-cell receptor**, and a **soluble** class II **MHC** molecule DR1, and the three binary complexes TCR-SEB, SEB-DR1, and the peptide-specific complex DR1-TCR. We report here that in each case the specificity of the **interaction** among the soluble molecules is the same as observed in biological assays. Native gel electrophoresis and plasmon resonance affinity measurements indicate that SEB-TCR complex can form in the absence of class II **MHC** and that SEB-TCR **interaction** increases the **binding** of DR1. The observation that a superantigen can form complexes with TCR in both the absence and presence of class II **MHC** may provide a mechanism for its ability to induce anergy in some circumstances and activation in others (reviewed in ref. 8).

Binary and ternary complexes between T-cell receptor, class II **MHC** and superantigen in vitro.



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08604495 95293021 PMID: 7774629

Soluble mouse major histocompatibility complex class II molecules produced in Drosophila cells.

Wallny H J; Sollami G; Karjalainen K

Basel Institute for Immunology, Switzerland.

European journal of immunology (GERMANY) May 1995, 25 (5)

p1262-6, ISSN 0014-2980 Journal Code: 1273201

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

We have exploited *Drosophila melanogaster* Schneider cells and compatible inducible expression vectors to produce large amounts of secreted major histocompatibility complex (MHC) class II molecules (I-E<sub>d</sub>). A simple two-step purification protocol was developed. In the first step, recombinant molecules were enriched using a monoclonal anti-class II antibody column followed by a nickel chelate column which further purified and concentrated the recombinant protein to several mg/ml. Characterization of the purified material indicates that the molecules are correctly assembled into alpha beta heterodimers. Further analysis shows that the recombinant MHC class II molecules are devoid of endogenous peptides and, therefore, homogeneous peptide/MHC complexes could be prepared by adding exogenous I-E<sub>d</sub>-specific peptides at slightly acidic pH. Upon peptide addition, molecules underwent a conformational change into a more compact form revealed by gel filtration analysis. In addition, the peptide/MHC complexes were biologically active. As little as 10 ng of these complexes coated on plastic from a 100 ng/ml solution were sufficient to trigger antigen-specific T cell hybridomas. These MHC class II molecules, together with various forms of soluble T cell receptor (TCR) proteins, provide valuable tools to analyze the molecular details of TCR/antigen recognition.

May 1995,

... cells and compatible inducible expression vectors to produce large



18/3,K,AB/5 (Item 5 from file: 155)  
DIALOG(R)File 155:MEDLINE(R)  
(c) format only 2004 The Dialog Corp. All rts. reserv.

10432813 96239290 PMID: 8656044

Soluble extracellular antigen-specific T cell immunoproteins.

Cone R E

Department of Pathology, University of Connecticut Health Center,  
Farmington, 06030-3105, USA.

Journal of leukocyte biology (UNITED STATES) May 1996, 59 (5)  
p605-12, ISSN 0741-5400 Journal Code: 8405628

Document type: Journal Article; Review; Review, Tutorial

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Some T cells release proteins that **bind** specifically to antigens that have not been processed by antigen-presenting cells. These soluble immunoproteins induce or effect antigen-specific T cell functions in immunoregulation and/or hypersensitivity. After certain immunization regimens, T cell immunoproteins specific for the immunogen rise in serum, and therefore may be an antigen-specific, humoral manifestation of the activation of some T cells during an immune response. Although non-MHC (major histocompatibility complex)-associated antigen is bound, soluble antigen-specific T cell immunoproteins share variable and constant region epitopes and some amino acid sequence with the T cell receptor for antigen alpha or beta chains, and their expression depends on T cell receptor structural genes. Herein, the properties of extracellular antigen-specific T cell immunoproteins are reviewed and it is suggested that these molecules are a **soluble** analogue of the **T cell receptor** for antigen and provide an amplifying element for some T cell functions.

May 1996,

Some T cells release proteins that **bind** specifically to antigens that have not been processed by antigen-presenting cells. These soluble immunoproteins...



18/3,K,AB/4 (Item 4 from file: 155)  
DIALOG(R)File 155:MEDLINE(R)  
(c) format only 2004 The Dialog Corp. All rts. reserv.

10483985 96293497 PMID: 8692966

Covalent assembly of a **soluble T cell receptor**  
-peptide-major histocompatibility class I complex.

Gregoire C; Lin S Y; Mazza G; Rebai N; Luescher I F; Malissen B  
Centre d'Immunologic, Institut National de la Sante et de la Recherche  
Medicale, Marseille, France.

Proceedings of the National Academy of Sciences of the United States of  
America (UNITED STATES) Jul 9 1996, 93 (14) p7184-9, ISSN  
0027-8424 Journal Code: 7505876

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

We used stepwise photochemical cross-linking for specifically assembling soluble and covalent complexes made of a T-cell antigen receptor (TCR) and a class I molecule of the major histocompatibility complex (MHC) bound to an antigenic peptide. For that purpose, we have produced in myeloma cells a single-chain Fv construct of a TCR specific for a photoreactive H-2Kd-peptide complex. Photochemical cross-linking of this TCR single-chain Fv with a soluble form of the photoreactive H-2Kd-peptide ligand resulted in the formation of a ternary covalent complex. We have characterized the soluble ternary complex and showed that it reacted with antibodies specific for epitopes located either on the native TCR or on the Kd molecules. By preventing the fast dissociation kinetics observed with most T cell receptors, this approach provides a means of preparing soluble TCR-peptide-MHC complexes on large-scale levels.

Covalent assembly of a **soluble T cell receptor**  
-peptide-major histocompatibility class I complex.



543711 96355329 PMID: 8702739

Expression of recombinant HLA-DR2 molecules. Replacement of the hydrophobic transmembrane region by a leucine zipper dimerization motif allows the assembly and secretion of soluble DR alpha beta heterodimers.

Kalandadze A; Galleno M; Foncerrada L; Strominger J L; Wucherpfennig K W  
Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts 02115, USA.

Journal of biological chemistry (UNITED STATES) Aug 16 1996, 271

(33) p20156-62, ISSN 0021-9258 Journal Code: 2985121R

Contract/Grant No.: CA47554; CA; NCI

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Major histocompatibility complex (MHC) class II molecules are membrane-anchored heterodimers that present peptides on the surface of antigen presenting cells to T cells. Soluble HLA-DR2 molecules were expressed for structural and functional characterization of the MHC/peptide/T cell receptor recognition unit. The alpha and beta chains of DR2 (encoded by the DRA, DRB1\*1501 genes) did not assemble in mammalian or insect cell lines when the transmembrane regions of one or both chains were truncated. The hydrophobic transmembrane regions of DRalpha and DRbeta facilitate assembly of the heterodimer and were therefore replaced by the leucine zipper dimerization motifs from the transcription factors Fos and Jun, which assemble as a soluble, tightly packed coiled coil structure. The DRalpha-Fos and DRbeta-Jun constructs were expressed in a methyaltrophic yeast, Pichia pastoris, using the alpha-mating factor secretion signal to direct expression to the secretory pathway. DR alphabeta heterodimers were purified from supernatants using an antibody specific for the DR alphabeta heterodimer. Kinetic and quantitative peptide binding experiments demonstrated that recombinant DR2 molecules were efficiently loaded with an antigenic peptide. Soluble DR2 molecules can be used to define structural aspects of the MHC/peptide/T cell receptor interaction and to study the signals induced by T cell receptor recognition of soluble DR2.peptide complexes.



Dialog Acc No: 2791527 IFI Acc No: 9631253

Document Type: C

METHOD FOR DETECTION OR QUANTITATION OF AN ANALYTE USING AN ANALYTE  
DEPENDENT ENZYME ACTIVATION SYSTEM; REACTING WITH A CONJUGATE CONSISTING OF  
A DETECTABLY LABELED SUBSTRATE SPECIFIC FOR ENZYME SYSTEM; DEPOSITS  
WHEREVER RECEPTOR IS IMMOBILIZED

Inventors: Bobrow Mark N (US); Litt Gerald J (US)

Assignee: Du Pont de Nemours, E I & Co

Assignee Code: 25048 Document Type: REASSIGNED

Publication (No,Date), Applic (No,Date):

US 5583001 19961210 US 94238186 19940504

Publication Kind: A

Calculated Expiration: 20131210

(Cited in 003 later patents)

Continuation Pub(No),Applic(No,Date): ABANDONED

US 92914374

19920715

Cont.-in-part Pub(No),Applic(No,Date): ABANDONED

US

89330357 19890329; ABANDONED

US 90494226

19900320

Division Pub(No),Applic(No,Date): US 5196306

US 90589719

19900928

Priority Applic(No,Date): US 94238186 19940504; US 92914374 19920715;

US 89330357 19890329; US 90494226 19900320; US 90589719 19900928

Abstract: The present invention concerns a method to catalyze reporter  
deposition to improve detection or quantitation of an analyte in a sample  
by amplifying the detector signal which comprises reacting an analyte  
dependent enzyme activation system with a conjugate consisting of a  
detectably labeled substrate specific for the enzyme system, said conjugate  
reacts with the analyte dependent enzyme activation system to form an  
activated conjugate which deposits substantially wherever receptor for the  
activated conjugate is immobilized, said receptor not being reactive with  
the analyte dependent enzyme activation system. In another embodiment the  
invention concerns an assay for detecting or quantitating the presence or  
absence of an analyte in a sample using catalyzed reporter deposition to  
amplify the reporter signal.

Publication (No,Date), Applic (No,Date):

...19961210

Non-exemplary Claims: ...5. An assay according to claim 1 wherein the  
**detectable label** is selected from the group consisting of  
enzymes, radioactive isotopes, fluorogenic, chemiluminescent, or  
electrochemical or...

...10. An assay according to claim 1 wherein the activated conjugate in  
step (c) is **biotinylated** and is reacted with a detectably labeled  
**streptavidin**.

...

...17. An assay according to claim 13 wherein the **detectable  
label** is selected from the group consisting of enzymes,  
radioactive isotopes, fluorogenic, chemiluminescent, or electrochemical  
or...

...19. An assay according to claim 13 wherein the activated conjugate step  
(b) is **biotinylated** and is reacted with a detectably labeled  
**streptavidin**.

9/3,K,AB/6

DIALOG(R) File 340:CLAIMS(R)/US Patent

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Dialog Acc No: 2730385 IFI Acc No: 9614951

Document Type: C



BETA 5 PROTEIN AND DNA ENCODING THE SAME; CONTACTING BIOLOGICAL SPECIMEN WITH ANTIBODY PROBE CAPABLE OF BINDING PROTEIN B5, DETECTING THE PRESENCE OF B5 BY DETERMINING WEATHER BINDING HAS OCCURED

Inventors: Hemler Martin E (US); Ramaswamy Hemavathi (US)

Assignee: Dana-Farber Cancer Institute Inc

Assignee Code: 11804

Publication (No,Date), Applic (No,Date):

US 5527679      **19960618**    US 9354077      19930427

Publication Kind: A

Calculated Expiration: 20130618

Division Pub(No),Applic(No,Date): ABANDONED

US 91694314

19910501

Priority Applic(No,Date): US 9354077      19930427; US 91694314      19910501

Abstract: In accordance with the present invention, a cDNA clone encoding a new human Beta subunit which was designated Beta 5 was found. Probes for this nucleotide sequence are described. In addition, the Beta 5 protein, its associated subunit and its cell distribution were characterized. In another embodiment, this invention relates to assays for detecting this protein. Beta 5 subunit was found present on carcinomas, but absent from lymphoid cells. Consequently, this protein can be used to determine the presence of carcinoma.

Publication (No,Date), Applic (No,Date):

...**19960618**

Non-exemplary Claims: ...7. An assay according to claim 5, wherein the

**detectable-label** is selected from the group consisting of radioisotopes, enzymes, fluorogenic, chemiluminescent, and electrochemical materials...

...assay according to claim 5, wherein the biotin-conjugated antibody is detected by reacting the **biotinylated** complex first with **streptavidin**-horseradish peroxidase followed by reaction orthophenylenediamine...

?



Document Type: C

COMPOSITIONS AND METHODS FOR THE DETECTION, QUANTITATION AND PURIFICATION OF ANTIGEN-SPECIFIC T CELLS; STABLE MULTIMERIC COMPLEXES OF MAJOR HISTOCOMPATIBILITY PROTEINS AND SPECIFIC ANTIGENIC PEPTIDES TO BIND AND LABEL; MEDICAL DIAGNOSIS; AUTOIMMUNE DISEASE; ANTITUMOR, -CARCINOGENIC, AND ARTHRITIC AGENTS

Inventors: Altman John D (US); Davis Mark M (US); McHeyzer-Williams Michael G (US)

Assignee: Stanford, Leland Jr University Trustees

Assignee Code: 49136

Publication (No,Date), Applic (No,Date):

US 5635363. 19970603 US 95396220 19950228

Publication Kind: A

Calculated Expiration: 20150228

(Cited in 003 later patents)

Priority Applic(No,Date): US 95396220 19950228

Abstract: T cells are specifically labeled according to their antigen receptor by binding of a multimeric binding complex. The complex is prepared with major histocompatibility complex protein subunits having a homogeneous population of peptides bound in the antigen presentation site. The multimeric MHC-antigen complex forms a stable structure with T cells, thereby allowing for the labeling, identification and separation of specific T cells.

Publication (No,Date), Applic (No,Date):

...19970603

Non-exemplary Claims: ...method according to claim 3, wherein said modifying enzyme is BirA, said fusion protein is **biotinylated**, and said multivalent entity comprises at least one of **streptavidin** and avidin...

...5. A method according to claim 4, wherein said multimeric binding complex further comprises a **detectable label**.

...

...method according to claim 8, wherein said modifying enzyme is BirA, said fusion protein is **biotinylated**, and said multivalent entity comprises at least one of **streptavidin** and avidin...

...A method according to claim 9, wherein said multimeric binding complex further comprises a light **detectable label**.

...

...complex according to claim 14, wherein said modifying enzyme is BirA, said fusion protein is **biotinylated**, and said multivalent entity comprises at least one of **streptavidin** and avidin...

...binding complex according to claim 15, wherein said multimeric binding complex further comprises a light **detectable label**.

9/3,K,AB/4

DIALOG(R) File 340:CLAIMS(R)/US Patent

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Dialog Acc No: 2803363 IFI Acc No: 9701036

Document Type: C

METHOD FOR LABELING DNA BY RING-OPENING OF PURINE BASES; DIRECTLY THROUGH A LINKING GROUP OR SUBSEQUENT DERIVITIZATION OF BLOCKING GROUP; DIAGNOSIS OF GENETIC DISORDERS

Inventors: McCabe Mead M (US)

Assignee: Miami, University of



Assignee Code: 55026

Publication (No,Date), Applic (No,Date):

US 5593829      19970114 US 94241385      19940503

Publication Kind: A

Calculated Expiration: 20140114

(Cited in 003 later patents)

Continuation Pub(No),Applic(No,Date): ABANDONED  
19911206

US 91802815



Set	Items	Description
? s biotinylat?		
S1	703	BIOTINYLAT?
? s detectable(5n) label		
	16234	DETECTABLE
	21874	LABEL
S2	2973	DETECTABLE(5N) LABEL
? s s1 and s2		
	703	S1
	2973	S2
S3	62	S1 AND S2
? s streptavidin		
S4	1178	STREPTAVIDIN
?		
? s fluorescent		
S5	22747	FLUORESCENT
? s s3 and s4		
	62	S3
	1178	S4
S6	29	S3 AND S4
? s s6 and s5		
	29	S6
	22747	S5
S7	8	S6 AND S5
? s s7 and py<1998		
	8	S7
	2952045	PY<1998
S8	0	S7 AND PY<1998
? s s6 and py<1998		
	29	S6
	2952045	PY<1998
S9	6	S6 AND PY<1998
? t s9/3,k,ab/1-6		

9/3,K,AB/1  
 DIALOG(R)File 340:CLAIMS(R)/US Patent  
 (c) 2004 IFI/CLAIMS(R). All rts. reserv.

Dialog Acc No: 2919117 IFI Acc No: 9736476  
 Document Type: C  
 METHODS FOR SYNTHETIC UNRANDOMIZATION OF OLIGOMER FRAGMENTS; DETERMINATION OF TARGET PROPERTY; BIOSYNTHESIS CYCLES  
 Inventors: Anderson Kevin (US); Brown-Driver Vickie (US); Bruce Thomas W (US); Cook Phillip Dan (US); Davis Peter (US); Ecker David J (US); Freier Susan M (US); Hanecak Ronnie (US); Sanghvi Yogesh S (US); Vickers Timothy (US); Wyatt Jacqueline (US)  
 Assignee: ISIS Pharmaceuticals Inc  
 Assignee Code: 28846  
 Publication (No,Date), Applic (No,Date):  
 US 5698391 19971216 US 94357396 19941216  
 Publication Kind: A  
 Calculated Expiration: 20141216  
 Document Type: CERTIFICATE OF CORRECTION Certificate of Correction Date: 19990119  
 Cont.-in-part Pub(No),Applic(No,Date): ABANDONED US  
 91749000 19910823; US 94196103 19940222  
 Priority Applic(No,Date): US 94357396 19941216; US 91749000 19910823;  
 US 94196103 19940222  
 Disclaimer Date: 20140222

Abstract: Methods useful for the determination of oligomers which have specific activity for a target molecule from a pool of primarily randomly assembled oligomers are provided. The disclosed methods involve repeated



syntheses of increasingly simplified sets of oligomers coupled with selection procedures for determining oligomers having the highest activity. Freedom from the use of enzymes allows the application of these methods to any molecules which can be oligomerized in a controlled fashion.

Publication (No,Date), Applic (No,Date):  
...19971216